MOLECULAR AND CELLULAR BIOLOGY, Aug. 1996, p. 4495–4503 Vol. 16, No. 8
0270-7306/96/$04.00+0

Germ Line-Specific Expression of Intracisternal A-Particle Retrotransposons in Transgenic Mice

ANNE DUPRESSOIR AND THIERRY HEIDMANN*

Unité de Physicochimie et Pharmacologie des Macromolécules Biologiques, CNRS URA 147, Institut Gustave Roussy, 94805 Villejuif Cedex, France

Received 9 February 1996/Returned for modification 1 April 1996/Accepted 23 April 1996

Intracisternal A-particle (IAP) sequences are endogenous retrovirus-like mobile elements, or retrotransposons, present at 1,000 copies in the mouse genome. These elements transpose in a replicative manner via an RNA intermediate and its reverse transcription, and their transposition should therefore be tightly controlled by their transcription level. To analyze the in vivo pattern of expression of these retrovirus-like elements, we constructed several independent transgenic mice with either a complete IAP element marked with an intron or with the IAP promoter, or long terminal repeat (LTR), alone controlling the expression of a *lacZ* reporter gene with a nuclear localization signal. For all transgenic lines analyzed, IAP expression as determined by reverse transcription-PCR analysis was found to be essentially restricted to the male germ line. Furthermore, in situ 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) staining of all organs disclosed specific β-galactosidase-positive blue cells only within the testis, found as patches along the seminiferous tubules and often organized as assemblies of 2, 4, 8, or 16 cells. Histochemical analyses of tissues from 13.5-day-old embryos to adults demonstrated that this LTR activity is restricted to gonocytes and premeiotic undifferentiated spermatogonia. Finally, analysis of the methylation status of both transgenes and endogenous IAP LTRs demonstrated identical patterns, with methylation in somatic tissues and hypomethylation in the testis. Transgenic mice therefore reveal an intrinsic, highly restricted IAP expression which had escaped detection in previous global Northern (RNA) blot analyses and with possible strong biological relevance, as IAP activation specifically within the germ line might be a way to generate diversity at the evolutionary level without being deleterious to individuals.

Intracisternal A-particle (IAP) sequences are moderately reiterated transposable elements (approximately 1,000 copies in the mouse genome) which are closely related to retroviruses and transpose via the reverse transcription of an RNA intermediate (23, 30). They possess 5′ and 3′ long terminal repeats (LTRs) flanking *gag-pol* open reading frames (30, 42) and encode particles which are intracellular and not infectious. Several classes of IAP elements have been identified, and they differ essentially by internal deletions of various lengths and/or one specific insertion (36, 51). IAPs most probably derive from an ancient retrovirus that invaded the mouse genome and has now reached a status and life cycle compatible with, and possibly beneficial to, the host, as observed in several host-parasite associations and as extensively characterized for *Drosophila* species (reviewed in reference 38).

These elements are not inactive, as at least some of them can still transpose. Several examples of germ line and somatic IAP transpositions (in the latter case mostly within cell lines) have been reported; they were identified as a result of either the inactivation or the activation of the genes close to which they have inserted (reviewed in references 30 and 31; see also references 13, 14, and 40 and references therein). IAP transposition can be a major source of mutations and as such contribute to the induction and/or enhancement of pathological processes (13, 14, 30, 31, 40). In fact, enhanced IAP expression and particle production is a common aberration of tumor cells, both from transplanted tumors and from established cell lines, including myelomas, neuroblastomas (32), and some cell lines treated with carcinogenic agents (26, 27).

IAP expression in vivo has been studied primarily by Northern (RNA) blot analysis of whole tissues. During early embryogenesis, IAP transcripts can be detected in embryos from the first cleavage to blastocysts (46, 56); in adult mice, IAP transcripts can be detected at significant levels in several somatic tissues (15, 32; reviewed in reference 30), with highest expression in the cortical thymocytes (but still <5% of that in myeloma cells). This expression, however, varies among different mouse strains, and in several instances IAP proteins or particles could not be detected by using immunological approaches (32, 34) or electron microscopy (53). The biological relevance of these transcripts, therefore, can be questioned. For instance, it is not known whether they are associated with a low level of expression from the whole population of IAP sequences or whether they correspond to a more clonal expression from a limited number of elements, possibly activated by a position effect. Previous studies tend to suggest (see also Discussion) that the second hypothesis is the most plausible, since sequencing of cDNA generated from RNA of normal tissues, including the thymus, or from tumors resulted in only a limited number of IAP subtypes (35, 41). This interpretation would be consistent with the requisite for genetic stability, particularly as a large fraction of the genomic IAP sequences are noncoding as a result of internal deletions within the IAP open reading frames or point mutations (therefore accounting for the absence of IAP proteins despite the presence of transcripts [see above]). Accordingly, a significant fraction of the IAP transcripts could originate from unique sequences, possibly without coding capacities, activated by a position effect, which could mask a still unknown and biologically more relevant activity.

To get insight into the intrinsic regulatory properties of the

* Corresponding author. Mailing address: Unité de Physicochimie et Pharmacologie des Macromolécules Biologiques, CNRS URA 147, Institut Gustave Roussy, 39 rue Camille Desmoulins, 94805 Villejuif Cedex, France. Fax: 33/1 46 78 41 20. Electronic mail address: heidmann@igr.fr.

IAP LTR in vivo and identify the cells in which they might be active, independent of possible position effects, we have generated transgenic mice and analyzed the pattern of expression of IAP elements marked with reporter genes. Transgenes containing the 5′ LTRs of two different IAP elements and a gene for a nucleus-targeted β-galactosidase allowing a cell-by-cell localization of expression (LTRlacZ transgenes), as well as a transgene with a complete IAP element marked with an intron-containing cassette, were constructed. Interestingly, in all of the independent transgenic lines that were established, the IAP LTR directed expression of the transgenes in the male germ line. More precisely, in situ 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) staining of samples from the LTRlacZ mice demonstrated expression at premeiotic stages, in the gonocytes and the undifferentiated spermatogonia. Expression of the transgenes was found to be position and reporter gene independent and therefore should reflect an intrinsic property of the IAPs that had previously escaped detection by global approaches. Restriction with methylation-sensitive enzymes further demonstrated methylation of both the transgene and the endogenous IAP sequences in the somatic tissues and hypomethylation in the testis. Overall, these data suggest the existence of complex regulatory mechanisms, including methylation, that allow IAP transposition when it might generate diversity (i.e., in germ cells) and repress it when it would be deleterious for the individual (i.e., in somatic cells).

MATERIALS AND METHODS

DNA constructs. The IAP–interleukin 3 (IAP-IL3) LTR without the IL3 gene flanking sequence was obtained from a transposed IAP-IL3 sequence subcloned into the *Eco*RV site of pSK$^-$ (p5-2G [23]). The complete p(IL3-LTRlacZ) transgene was constructed by a three-fragment ligation between a 367-bp *Aat*II-*Pvu*II restriction fragment containing the 3′ part of the 5′ LTR plus 150 bp of internal untranslated sequences from IAP-IL3 (55), a 4.5-kb *Sal*I-*Xho*I fragment blunt ended at the *Sal*I site and containing the nls*lacZ* gene (see Results) fused to the simian virus 40 intron and polyadenylation signal (excised from plasmid pTZ nls lacZ, a gift from C. Bonnerot and J.-F. Nicolas), and the p5-2G vector opened at the homologous *Aat*II site within the LTR and at the *Sal*I site within the pSK$^-$ polylinker. To generate p(MIA14-LTRlacZ) with non-IAP sequences as in p(IL3-LTRlacZ), a PCR fragment was generated by using primer m1 (5′-CGTGTGAGTACCTTATCCTTAGAATCCAGAAGAGGTGTTGGGAG CCGCGCCCAC), containing the 35 bp of flanking DNA in p(IL3-LTRlacZ) fused to the first 19 bp of the 5′ LTR of MIA14 (42), and primer m2 (5′-CTGAAAAGTTCTGAATTCATACAG), corresponding to a 24-bp internal sequence from the *Pvu*II site in MIA14 homologous to that in IAP-IL3 (position 489 in reference 42). The 525-bp fragment obtained upon PCR amplification using pMIA14 as a template was cloned into p(IL3-LTRlacZ) in place of the IAP-IL3 sequence upon restriction and Klenow treatment of the vector by *Spe*I and *Sal*I. The intron-containing complete IAP transgene was constructed by using the p(IL3-LTRlacZ) transgene upon subcloning of the LTR as a *Sac*I-*Ban*I (in the pSK$^-$ polylinker and 3 bp 3′ to U5, respectively) blunt-ended fragment into the *Pvu*II and *Bgl*II blunt-ended sites of the ppolyIIId (33) polylinker. Reconstitution of the complete IAP-IL3 sequence was then achieved upon insertion of the *Mlu*I-*Mlu*I IAP-IL3 fragment (*Mlu*I unique in the IAP LTR) into the homologous *Mlu*I site of the subcloned LTR. The reconstituted IAP-IL3 sequence was opened at the unique internal *Sal*I site, and a reduced *Mlu*I-*Sal*I fragment from the neoRT indicator gene (23, 24) lacking the internal *tk* promoter was inserted, after blunt ending of both vector and insert, resulting in the intron-containing complete IAP transgene. All transgenes were excised as *Sac*II-*Xho*I fragments, which were purified by agarose gel electrophoresis and electroelution prior to microinjection into fertilized eggs.

Transgenic mice. Approximately 500 copies of DNA fragment were microinjected into fertilized eggs resulting from mating between (C57BL/6 × DBA/2) hybrids, using established procedures (25). Transgenic mice were identified by Southern blotting experiments using 10 μg of DNA from the tails of 3-week-old mice (data not shown). Southern blots (Biohylon Z+ membrane; Bioprobe) were probed with 15 ng of random-primed ^{32}P-labeled *lacZ* or *neo* fragments (Multiprime kit; Amersham) in Church solution (7% sodium dodecyl sulfate [SDS], 1 mM EDTA [pH 8], 0.5 M $NaPO_4$ [pH 7]) without bovine serum albumin (BSA). Filters were washed twice for 15 min at 65°C in 0.5 × SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% SDS. Transgene copy number was determined by densitometric analysis of the autoradiograms (using preflashed films) or by BioImager analysis. Transgenic lines were established by mating founder mice with DBA/2 or (C57BL/6 × DBA/2) mice.

β-Galactosidase histochemical analysis. Transgenic embryos were obtained from hemizygous transgenic males mated with (C57BL/6 × DBA/2) females induced to superovulate; the day the copulation plug was observed was designated 0.5 day postcoitum (p.c.). The procedure for in situ detection of β-galactosidase was identical for pre- and postimplantation embryos and for adult organs except for the fixation step. Briefly, fixation (whole mount) was with 4% paraformaldehyde in phosphate-buffered saline (PBS) at 4°C for 5 min with preimplanation embryos and 1 to 2 h for postimplantation embryos. Adult mice were intracardiacally perfused with 20 ml of PBS followed by 20 ml of fixative; organs were then removed and placed in fixative for 15 min. Fixed embryos and tissues were washed with PBS at 4°C for 15 min to 2 h, depending on their size. Staining was then carried out overnight at 30°C as described previously (49), using 0.4 mg of X-Gal per ml. After staining, tissues were rinsed with PBS and kept in 4°C in a 30% sucrose–PBS solution until being photographed as whole mounts; they were thereafter embedded in TissuTek and frozen in isopentane cooled with dry ice. Sections (6 μm) were cut on a cryostat at −22°C and counterstained with hematoxylin and eosin according to standard procedures.

Reverse transcription-PCR (RT-PCR) analysis. Total RNAs were extracted from the frozen organs by the guanidine thiocyanate-CsCl method (7). Twenty micrograms of total RNA was reverse transcribed from a poly(dT) primer [or random (dN)6 primers for the IAPn mice] in 30 μl of reaction medium containing 0.5 mM each deoxynucleoside triphosphate, 3 μg of BSA, 10 mM dithiothreitol, oligo(dT) (100 μg/ml) or (dN)6 (2 mM), 20 U of RNAsin, 50 mM Tris-HCl, 75 mM KCl, 3 mM $MgCl_2$, and 600 U of Moloney murine leukemia virus reverse transcriptase (Gibco BRL). Reactions were carried out for 60 min at 37°C. Then 3 μl of the reverse transcription reaction mixture was amplified in 50 μl of PCR medium containing 10 mM Tris HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% (wt/vol) gelatin, 0.02 μM primers, and 5 U of *Taq* polymerase (Amersham). After an initial step at 94°C (2 min), a 30- to 35-cycle amplification was performed (10 s at 60°C, 70 s at 72°C, 10 s at 94°C) and ended with one terminal cycle (10 s at 60°C, 7 min at 72°C). The primers were t1 (5′-CAGCTGCTGCC TGAGGCTGGACG), t2 (5′-GGAGGAGTAGAATGTTGAGAGAGTCAGC), t3 (5′-CGGCATCAGAGCAGCCGATTGTCTG), and t4 (5′-GGCCTCGAA CACCGAGCGACCCTG).

Analysis of methylation status. Organs were lysed overnight in 50 mM Tris (pH 8)–10 mM EDTA (pH 8)–100 mM NaCl–0.2% SDS–500 μg of proteinase K per ml; DNA was then extracted by phenol-chloroform treatment and ethanol precipitation. For each restriction analysis, 30 μg of DNA was cleaved with at least a fourfold excess of restriction enzyme for 10 h. λ DNA was added at a 10:1 (genomic:λ) DNA ratio to monitor the extent of the digestion. Restriction DNA were phenol-chloroform extracted, electrophoresed on a 1% agarose gel, and then analyzed by Southern blotting (see above). To monitor the methylation of the transgene, a 600-bp *Hin*cII-*Hin*cII fragment isolated from plasmid pGEMnlslacZ was first used as a probe (probe I; see Fig. 5); after dehybridization, the same blot was then rehybridized with a *Pvu*II-*Xba*I fragment isolated from IAP-IL3 plasmid (probe II; see Fig. 5).

RESULTS

Establishment of transgenic mouse lines. Two series of transgenic mice were generated with DNA constructs containing the LTR and part of the 5′ untranslated sequence of IAP elements, the nls*lacZ* reporter gene coding for a β-galactosidase with a nuclear location signal (4), and at the 3′ end the intron and polyadenylation signal from the simian virus 40 large T antigen (Fig. 1A). LTRs from two representative IAP sequences were used: from IAP-IL3 (55), from a class IΔ1 IAP sequence, which had inserted within the IL3 gene and was further demonstrated to be still transposition competent in an ex vivo assay (23), and from MIA14 (42), a full-length class I genomic IAP sequence. The LTRs from these two sequences are closely related (Fig. 1A), as both contain previously identified regulatory sites for Sp1 and YY1 (50), a glucocorticoid-responsive element (GRE), two enhancer sequences (Enh1 and Enh2 for the previously identified EBP 80 factor [17]), a cyclic AMP-responsive element (ATF/CRE), and CAAT and TATA boxes (reviewed in reference 30). Differences, however, exist (Fig. 1A), for instance, in the Enh2 sequence and in the TATA box domain, which displays a GAGAA motif in IAL-IL3, as observed for a large fraction of the members of the IAP family. To minimize the possible influence of adjacent cellular sequences, especially in the case of IAP-IL3 and which had inserted within the regulatory sequences of the IL3 gene, these sequences were substituted with <40-bp sequences unrelated to IL3 and identical for the two constructs (see Materials and Methods). Both constructs are functional, as they produce,

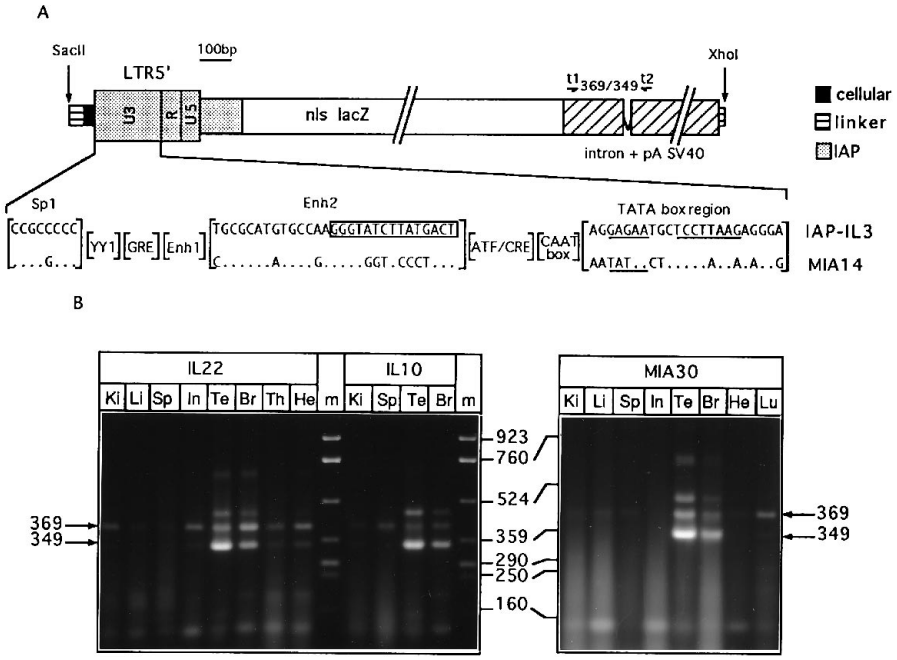


FIG. 1. (A) Structures of the IL3-LTRlacZ and MIA-LTRlacZ transgenes. The transgenes contain the 5′ LTR and part of the untranslated sequence from either IAP-IL3 or MIA14 (grey domain), the nls*lacZ* gene (open box), and the simian virus 40 (SV40) intron plus polyadenylation (pA) sequences (hatched domain); 35 bp of cellular sequence (dark) and linkers (striped) within the microinjected DNA are also represented. Essential sites within U3 are indicated for both transgenes, with the nucleotide sequences given when they are different; the motif common to recently transposed IAP elements within the Enh2 domain is boxed; putative TATA boxes are underlined. Small horizontal arrows represent the primers (t1 and t2) used for RT-PCR amplification of the spliced transcripts. The *Sac*II and *Xho*I sites used to excise the microinjected fragment are indicated. (B) RT-PCR analysis of transgenes expression in various organs. Total RNAs were reverse transcribed with an oligo(dT) primer, and resulting DNAs were PCR amplified with primers t1 and t2 (see panel A). Then 10-μl aliquots were loaded on 2% agarose gels, and fragments were visualized by UV ethidium bromide staining. Lane m, size markers. Fragments of expected sizes for spliced RNAs (349 bp) and for contaminating genomic DNA or unspliced RNA (369 bp) are indicated by arrows. Samples were from the testis (Te), brain (Br), heart (He), intestine (In), kidney (Ki), thymus (Th), spleen (Sp), liver (Li), and lung (Lu). RNAs were from either IL3-LTRlacZ (IL22 and IL10 lines) or MIA-LTRlacZ (MIA30 line) transgenic mice. PCR conditions: 0.6 μg of reverse-transcribed RNA and 33-cycle amplification for IL22; 2 μg of reverse-transcribed RNA and 30-cycle amplification for IL10 and MIA30.

when introduced by transfection into tumoral 3TDM1 cells, blue nuclei upon X-Gal histochemical staining (about 10 times more than a similar construct with the LTR from the Moloney murine leukemia retrovirus [data not shown]). We also generated another series of transgenic mice which contained the whole IAP-IL3 (i.e., both LTRs with minimal flanking DNA as described above and internal IAP sequences) marked with an intron-containing reporter gene as described in reference 23 (with deletion of the reporter internal promoter to prevent possible interference with the IAP-LTR [see Materials and Methods]). This transgene does not contain the *lacZ* gene, but its expression could still be assessed by RT-PCR (see below) using primers bracketing the reporter gene intron (Fig. 2A).

In all cases, the *Sac*II-*Xho*I fragments containing the transgene with minimal plasmid sequences were injected into the fertilized oocytes of B6D2 hybrid mice as described previously (25). Three founder mice for the IAP-IL3 (ILn mice), four founder mice for the MIA14 construct (MIAn mice), and two founder mice for the complete IAP construct (IAPn mice) were obtained. Lines were established from each by mating founder mice with DBA/2 or B6D2 mice (except for MIA61, which did not transmit the transgene). Southern blot analysis of genomic DNAs from the various transgenic lines with *lacZ* or *neo* probes, using enzymes which cut once in the transgene, showed that the transgene copy number ranged from 3 to ~200 (IL10, 6; IL18, 200; IL22, 60; MIA30, 3; MIA107, 20; MIC108, 80; IAP6, 2; IAP13, 20) with, as expected for independent transgenic lines, distinct patterns for the flanking genomic DNAs.

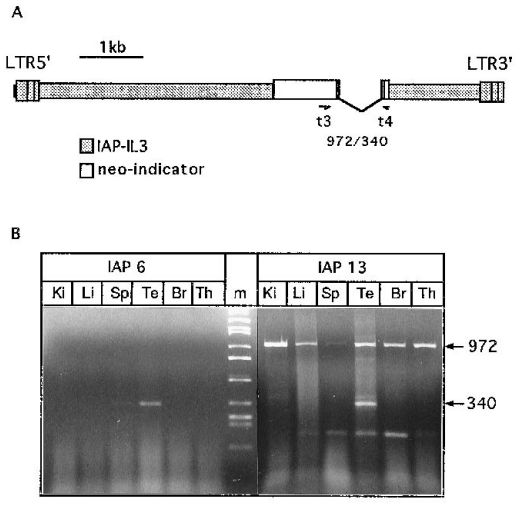


FIG. 2. (A) Structure of the intron-containing complete IAP transgene. The transgene contains the complete IAP-IL3 sequence (grey domain) and a neo-indicator gene (open box) derived from the previously described neoRT gene (23, 24) by elimination of the internal *tk* promoter. Intronic sequence of the reporter gene and primers (t3 and t4) used for RT-PCR amplification of the spliced transcripts are represented. (B) RT-PCR analysis of transgene expression in various organs. Total RNAs were reverse transcribed with random hexamer primers, and resulting cDNAs were PCR amplified with primers t3 and t4; 10-μl aliquots were loaded on 2% agarose gels. Abbreviations and marker (m) are the same as for Fig. 1B. Fragments of expected sizes for spliced RNAs (340 bp) and contaminating genomic DNA or unspliced RNA (972 bp) are indicated by arrows. PCR conditions: 30-cycle amplification for IAP6; 33-cycle amplification for IAP13.

Tissue-specific expression of the transgenes. To define the pattern of expression of the transgenes, total and poly(A)$^+$ RNAs were extracted from the liver, brain, testis, kidney, spleen, intestine, thymus, heart, and lung. In no instance could transgene expression be detected by Northern blot analysis (data not shown), and RT-PCR analyses were therefore performed (Fig. 1B and 2B). Total RNAs were reverse transcribed from oligo(dT) or (dN)6 random primers, and the resulting cDNAs were PCR amplified in one step, using primers (t1 and t2 or t3 and t4, respectively) bracketing the transgene intron (Fig. 1A and 2A). RNA-specific amplification (resulting in intronless 349- and 340-bp fragments for the LTRlacZ and the complete IAP constructs, respectively) should then be easily distinguished from that corresponding to contaminating genomic DNA or unspliced RNA (369 or 972 bp, respectively). As illustrated in Fig. 1B for two transgenic lines with the IL3-LTRlacZ transgene and one transgenic line with the MIA-LTRlacZ transgene, and in Fig. 2B for the two transgenic lines with the complete IAP transgene, RNA-specific amplification was observed in the testes, and to a smaller extent (at least 10-fold as determined by semiquantitative PCR [not shown]) in the brains of LTRlacZ mice. This severely restricted pattern of expression was observed for all of the transgenic lines that we had generated except the IL18 line, which did not express the transgene. It was stable in the descendants in all generations of mice examined (generations F_2 to F_5 were tested).

The tissue specificity of transgene expression was further delineated by an extensive histochemical analysis of β-galactosidase activity, upon in situ X-Gal staining of most tissues from the LTRlacZ transgenic mice. After intracardiac fixation, the following organs were assayed: spleen, kidney, liver, colon, intestine, testis, ovary, lymph nodes, muscles, heart, lung, thymus, brain, salivary gland, and bone marrow cells (flushed from both femurs). Consistent with the RT-PCR analysis, blue-stained nuclei were observed in the testes (Fig. 3) in all of the transgenic lines except line IL18; no blue cells were detected in the latter line, in agreement with the lack of expression in the RT-PCR analysis. The level of transgene expression, as assessed by both the extent and the intensity of X-Gal staining, disclosed individual quantitative variations, usually with a lower level in the MIA14 transgenic lines. For both the MIA- and IL3-LTR transgenic mice, the tissue-specific expression in the testis was maintained over generations (up to five generations tested). Finally, in the IL22 and MIA30 lines, some extra staining was detected in the brain; this staining, which was restricted to the F_1 generation (F_0 has not been examined), disappeared in the next generation without evidence for any difference in the pattern of transgene integrations (at least as revealed by Southern blot analysis) and was not further analyzed. No blue-stained nuclei were detected in any of the other organs tested or within embryos upon in toto X-Gal staining (except for the gonadal tissues [see below]).

Transgene expression in the testis is restricted to gonocytes and undifferentiated spermatogonia. One essential advantage of the in situ detection assay is that it allows a cell-by-cell analysis of transgene expression. Testes were first stained as whole mounts and then processed as histochemical sections. Examination of the adult testis disclosed nuclearly blue-stained cells (Fig. 3c to e) which appear as patches (among approximately 20 to 50 per testis) along the seminiferous tubule (Fig. 3c shows patches that can be detected without unwinding of the tubules). Staining is at the periphery of the seminiferous tubule, as evidence by cross sections (Fig. 3f to i). Stained cells are not interstitial cells (i.e., hormone-producing Leydig cells or myoid cells) but are internal to the tubules. Blue cells lie against the basement membrane and display oval nuclei of approximately 15 μm (Fig. 3h and i). On the basis of their morphology and position in the tubule, these blue cells can be identified as type A spermatogonia (48). An in toto examination of the labeled tubules further disclosed very characteristic configurations for these stained cells, allowing a more precise identification: they are either isolated or associated in chains of 2, 4, 8, or 16 cells (Fig. 3d and e). This topographic arrangement is typical for undifferentiated type A spermatogonia (11), which are the functional spermatogenic stem cells that can perform either self-renewing or proliferative divisions before differentiating into type A spermatogonia (Fig. 4). In the adult testis, no other blue cell could be detected at any other location. For instance, the type B spermatogonia, which derive from the type A spermatogonia and are clearly visible in the cross sections (Fig. 3h and i), never stained blue. This finding indicates that transgene expression is restricted to a definite and very early stage of spermatogenesis, in premeiotic cells before their entry into the differentiation pathway and their concomitant migration toward the center of the tubule, which will finally result in mature spermatozoa (Fig. 4).

We then examined whether transgene expression could be detected in the embryonic progenitors of the undifferentiated type A spermatogonia. Before birth, only two cell types are present in the seminiferous cords: small, immature Sertoli cells, which are always located at the periphery of the tubule, and large, round gonocytes that lie at the center of the tubule and which start to proliferate and migrate to the basement membrane 1 day after birth to generate the undifferentiated type A spermatogonia (1, 9, 29). In toto X-Gal staining of the testis of 19-day p.c. embryos disclosed isolated large blue cells that can be observed by transparency within the tubules (Fig. 3a) or in cryostat sections (Fig. 3j to l). These cells can clearly be identified as gonocytes by their morphology and their position in the tubule. When X-Gal analysis was performed on the seminiferous tubules of 1-day postnatal embryos, some mitotic figures of labeled cells could be observed (Fig. 3b; a similar configuration is described in reference 29), in agreement with the expected onset of gonocyte proliferation at this period. Positive blue cells have also been observed in the seminiferous cords of 16-day p.c. embryos (data not shown); no staining could be detected in earlier-stage embryos (in two-cell embryos [>60 embryos tested], in 3.5-day p.c. blastocysts, or in 13-day p.c. embroys) or in female germ cells at stages that are developmentally analogous (48) to those of blue male germ cells.

Therefore, IAP LTR expression appears to be restricted to the undifferentiated spermatogenic stem cells, i.e., the embryonic gonocytes and, in adult mice, the undifferentiated type A spermatogonia deriving from them. In embryonic testes, the number of blue gonocytes varies from one individual to the other but never are all gonocytes stained (Fig. 3j to l). Similarly, not all undifferentiated type A spermatogonia in adult testis are stained; moreover, blue cells are not randomly spread along the seminiferous tubule; rather, most of them are grouped in patches (Fig. 3c to e). Although not precisely quantifiable because of individual variations, the number of patches in the adult testis (ranging from 20 to 50) relative to the number of blue gonocytes in embryos could be compatible with the hypothesis that all blue cells in a patch are the daughter cells from a single gonocyte progenitor. Histological examination of the seminiferous tubules at the stained patches (11a) did not reveal any correlation between the occurrence of blue cells and particular stages of the cycle of the seminiferous epithelium as determined previously (45).

Methylation status of the transgenes and of the endogenous IAPs. Since previous studies have suggested that hypomethylation of CpG sites within the 5′ LTR is a prerequisite for IAP

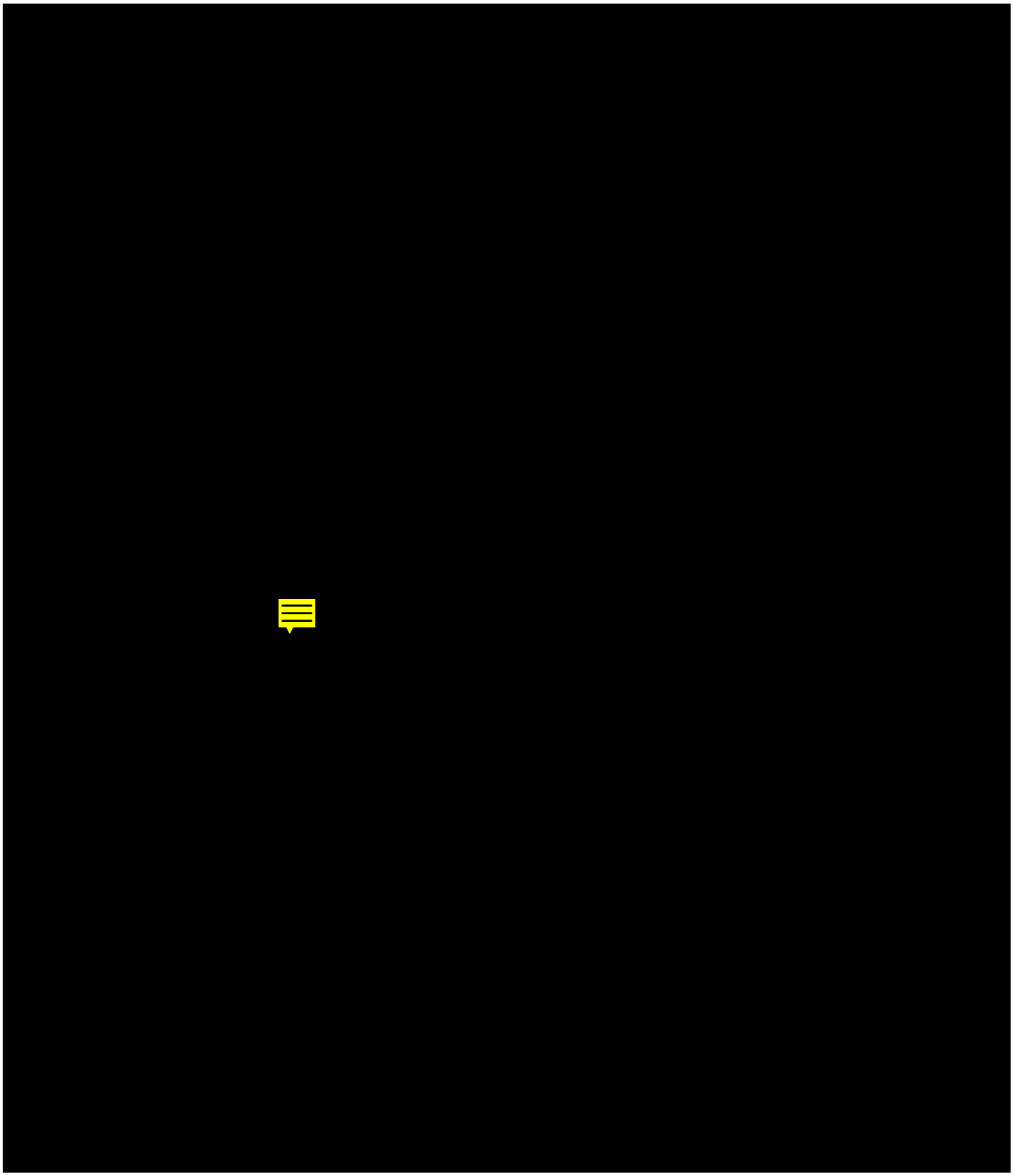


FIG. 3. Histochemical distribution of β-galactosidase in the seminiferous tubules of the IL22 transgenic line, in either whole mounts (a to e) or cryostat sections (f to l). Similar results were obtained with the other LTRlacZ-expressing lines. (a to e) Whole-mount X-Gal staining. (a) Part of a seminiferous cord of a 19-day p.c. embryo, disclosing (by transparency) an isolated blue cell (arrowhead) within the tubule; (b) part of the seminiferous tubule of a 1-day postnatal embryo with mitotic figures among blue cells (arrowhead); (c) adult testis after removal of the tunica albuginea (only peripheric tubules are visible), with transgene expression in patches (arrows); (d and e) unwound adult seminiferous tubules: stained blue cells localized at the surface of the tubules are either isolated or associated in chains of 2, 4, 8, or 16 cells (a schematic representation of the X-Gal pattern is shown in panel e). (f to l) Cryostat tubular sections of adult (f to i) and embryo (j to l) testes, counterstained with hematoxylin and eosin after X-Gal staining. The bars represent 15 μm except in panels f and j (50 μm). (f) Cross section of unwound testis disclosing blue cells only close to the periphery of the tubule (arrowheads to some of them). Staining is not observed in all tubules or within interstitial cells. (g to i) Higher magnifications show that labeled cells are localized against the internal side of the basement membrane but in an external position compared with type B spermatogonia (identified by their small nuclei strongly stained with hematoxylin [triangle in panel h]). Panels h and i show sections of expressing cell nuclei, approximately 15 μm in size and of oval shape. (j to l) Tubular section of 19-day p.c. embryo seminiferous tubules (hematoxylin and eosin counterstained after X-Gal staining), with blue-stained gonocytes with large round nuclei located at the center of the tubule and immature Sertoli cells with small pyramidal nucleus at the periphery (arrows in panel l).

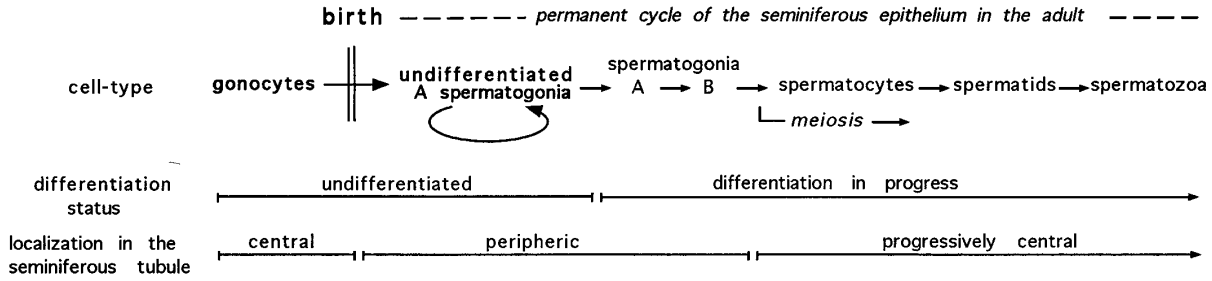


FIG. 4. Schematic representation of spermatogenesis in prenatal and adult mouse testes. In adults, spermatogenic cells progress in the permanent cycle of the seminiferous epithelium, from a self-renewing (circular arrow) stem cell population (the undifferentiated type A spermatogonia) that continuously provides differentiating spermatogonia, which are irreversibly committed to undertake the steps resulting in production of mature spermatozoa, through successive differentiation processes (small horizontal arrows) which result in the indicated germ cell types. Embryonic gonocytes (which stop proliferating from day 15 p.c. until birth) give rise to undifferentiated type A spermatogonia around birth. Localization of the various germ cell types in the seminiferous epithelium as well as the stage for the onset of meiosis (from references 1, 11, 29, and 48) are indicated.

expression (see Discussion), we investigated the methylation status of the transgene in the expressing organ (testis) and in weakly expressing (brain) or nonexpressing (kidney and liver) organs of the transgenic mice. The methylation-sensitive restriction sites usually monitored in the 5′ LTR (*Hha*I, *Hpa*II, or *Hae*II) could not be assessed with the construct used. However, one *Aat*II (GA^{m}CGTC) and one *Eag*I (^{m}CGGC^{m}CG) methylation-sensitive restriction site (37) are present in the region that has been shown to contain a functional ATF/CRE (Fig. 1A). This domain interacts, in vitro, with cellular nuclear factors and is responsible for the *ras*-mediated activation of the IAP LTR (18, 21); moreover, it has been established for other genes that CpG methylation of the CRE sequence abolishes transcriptional activation by preventing specific factor binding (28). To determine the methylation status of these sites, DNAs isolated from transgenic organs were cleaved first with *Hpa*I-*Xba*I, thus generating fragments of 1,150 bp, and then with one of the two methylation-sensitive enzymes; after electrophoresis, the blots were hybridized with probe I (Fig. 5). Occurrence of a 950-bp (*Aat*II-*Hpa*I) or 940-bp (*Eag*I-*Hpa*I) fragment should reflect the extent of demethylation of the *Aat*II or *Eag*I site, respectively; since *Eag*I is not active unless both CpG dinucleotides in its recognition sequence are demethylated, the amount of the *Eag*I-*Hpa*I fragment should be less than that of the *Aat*II-*Hpa*I fragment. As illustrated in Fig. 5, the 950-bp *Aat*II fragment is undetectable in the liver DNA, weak in the kidney and brain DNA, and much more intense in the testis DNA (in which its intensity reaches 15% of that of the 1,150-bp fragment). Accordingly, the 940-bp *Eag*I fragment is detected only in the testis. Therefore, at least the three CpG dinucleotides tested in the transgene LTR are less methylated in the testis than in the other organs. Actually, the extent of methylation at these sites is inversely correlated with transgene expression, since RT-PCR analysis performed on the same tissues (Fig. 1, line IL22), disclosed no signal in the liver or in the kidney, weak expression of the transgene in the brain, and strong expression in the testis.

To assess whether the LTRlacZ transgene is a reliable reporter of the methylation status of the endogenous IAPs, we examined the degree of methylation of the endogenous LTR at the same methylation-sensitive sites, taking advantage of a conserved *Xba*I site shared by most IAP sequences and located 330 bp downstream of the 5′ LTR (Fig. 5). The Southern blots were rehybridized with an IAP probe that had no sequence in common with the LTRlacZ transgene (probe II [Fig. 5]); if the *Aat*II or *Eag*I site is demethylated, specific *Xba*I-*Aat*II (540-bp) or *Xba*I-*Eag*I (530-bp) fragments should be detected (there is no *Hpa*I site upstream of the *Xba*I site). As illustrated in Fig. 5, the methylation status of the endogenous IAPs in the various organs actually correlates with that of the transgene: the 540-bp *Aat*II-specific fragment is much more intense in the testis than in other organs (and is even undetectable in the liver); accordingly, the 530-bp *Eag*I-specific fragment is detected only in the testis.

DISCUSSION

Identification of a niche for IAP expression. By using transgenes marked with an easily assayable reporter gene, allowing a cell-by-cell detection of transcriptional activity, we have unraveled a very restricted locus for expression of the mobile and potentially mutagenic murine IAP retrotransposons that had, rather expectedly, escaped detection in previous global Northern blot analyses. Among the six transgenic lines carrying the β-galactosidase gene under the control of the IAP-IL3 or MIA14 LTR that we have generated, five independent lines (two ILn lines and three MIAn lines) disclosed nuclearly blue cells upon in situ X-Gal staining only in the testis. Moreover, IAP LTR expression in this tissue was found to be restricted to precise and very early steps in spermatogenesis (in embryonic gonocytes and in adult undifferentiated type A spermatogonia) and to stop as soon as differentiation of these stem cells proceeds. This pattern of expression was stable over >5 generations and independent of the transgene integration site and should therefore be an intrinsic property of the IAP LTR. High-level expression of the LTRlacZ transgenes in this tissue was confirmed by a global RT-PCR analysis, whereas for one strain (IL18), absence of detectable activity in all tissues (upon both X-Gal staining and RT-PCR analysis) could simply suggest that the transgene has integrated into a region of inactive chromatin. Most importantly, a similarly restricted pattern of expression was found with another series of transgenic mice that we have generated, using as a transgene a complete IAP sequence containing both LTRs and internal IAP sequences and not containing the *lacZ* reporter gene. For the two independent lines obtained, the pattern of expression of this IAP, marked with an intron allowing an unambiguous RT-PCR analysis of the transcripts, was found to be similar to that for the LTRlacZ mice, therefore strongly suggesting (i) that the observed pattern of expression in the latter mice was not artifactually imposed by the *lacZ* reporter and (ii) that the essential *cis*-required elements for transcriptional regulation of the IAP promoter are included within the LTR and 5′ untranslated domain.

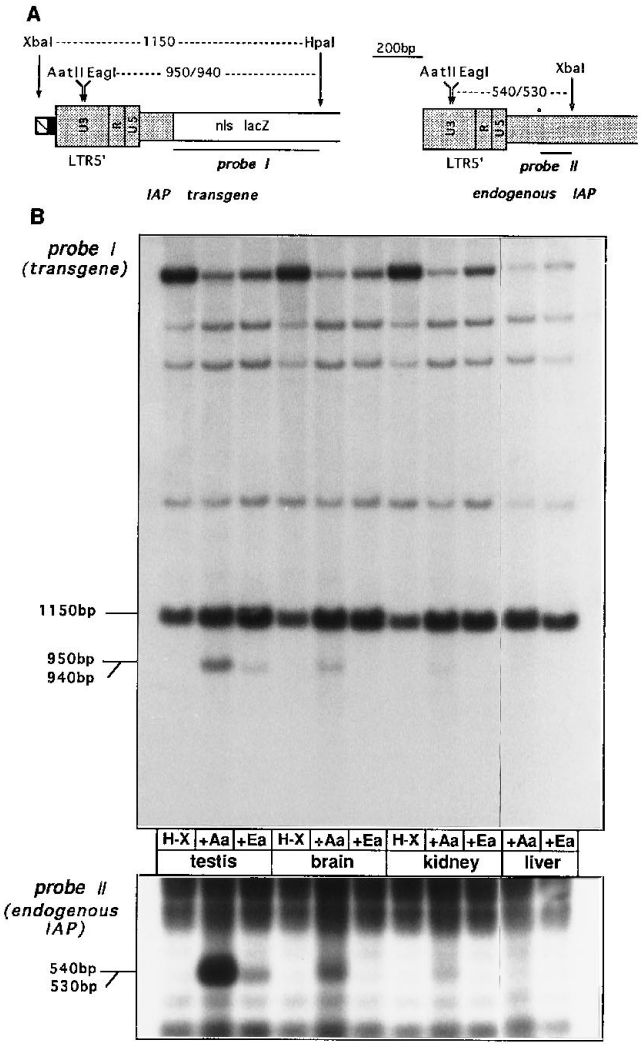


FIG. 5. Southern blot determination of the methylation status of the LTRlacZ transgene and of the endogenous IAPs in the testes, brains, kidneys, and livers of transgenic IL22 mice. (A) Structures of the transgene and of typical endogenous IAP sequences. Methylation-insensitive restriction sites (*Xba*I and *Hpa*I) and methylation-sensitive sites (*Aat*II and *Eag*I) are indicated, along with the lengths of the expected fragments (the *Aat*II and *Eag*I sites are 10 bp apart). Probe I (for the transgene) and probe II (for the endogenous IAPs) are represented by bars and do not overlap (the 3′ endpoint of IAP sequences in the transgene is indicated with a dot). (B) Southern blot analysis performed with 30 μg of genomic DNA from the indicated organs restricted with *Hpa*I plus *Xba*I alone (H-X) or with in addition *Aat*II (+Aa) or *Eag*I (+Ea); hybridization was with either probe I or probe II; the expected fragments and their sizes are indicated (the 940/950- and 530/540-bp bands were not visible in the liver even after fivefold-longer exposure).

Interestingly, we observed among the undifferentiated spermatogonia an unexpected mosaicism in LTRlacZ expression that was shared by independent lines and which therefore should not be a consequence of the transgene insertion sites. This patchy pattern of *lacZ* expression could result mainly from the mosaicism observed at the level of the gonocytes of the embryos (see Results) and might therefore reveal subtle and still unraveled differences in the level of transactivating factors and/or methylation state among this cell population in the seminiferous tubule. Mosaicism of expression in transgenic mice has been observed for other promoters in other cell types and in one case could be correlated to differences in methylation state among apparently identical cells (reference 39 and references therein).

The LTRlacZ transgene is, to our knowledge, the only *lacZ* marker specific for premeiotic undifferentiated male germ cells. This marker might therefore be of interest for all studies using these cells as a tool, such as spermatogonial cell transplantation (5). It might also be useful for studying in vivo the regulation of the proliferation and differentiation processes taking place in these stem cells, which are the only cells in the adult which are both self-renewing and totipotent. Driving the expression of genes such as c-*myc* or the large-T-antigen gene, the IAP LTR might allow the immortalization of germ cells at very early stages and their ex vivo manipulation.

Methylation of the retrovirus-like IAP sequences in vivo. It has been suggested that de novo methylation of foreign DNA could be a defense mechanism against the introduction of exogenous genetic information into the host chromosome which takes place, for instance, in the course of viral infection (see reference 12). Although IAP sequences can no longer be considered foreign material in mice (the transgenesis experiment may simply reproduce the original response to the ancestral intrusion of infectious IAP particles or of horizontally transferred nucleotidic material), we observed a massive de novo methylation of the IAP LTR in the somatic tissues. The methylation pattern of the transgene was demonstrated to parallel that of the endogenous IAPs. Analysis of the methylation status of the LTRlacZ transgene in expressing and nonexpressing tissues further revealed a correlation between tissue-specific expression and hypomethylation of the transgenic IAP LTR: the latter is heavily methylated in the liver and kidney, which do not express the transgene, only partially demethylated in the brain, in which low-level expression of the transgene could be detected by RT-PCR, and significantly demethylated in the testis, in which the transgene is the most heavily expressed. The possible effect of methylation on the in vivo expression of the transgene is consistent with a number of in vitro and ex vivo experiments suggesting that CpG methylation may regulate IAP transcription: in vitro methylation of the 5′ LTR inhibits the promoter activity in a transient transfection assay (19), IAP genes are hypomethylated in tumoral cell lines in which they are abundantly expressed (35, 43), and IAP expression can be induced upon cell treatment with the demethylating agent 5-azacytidine (26, 27). Although it is difficult to determine whether methylation is the cause or the consequence of the changes in gene expression, the present data suggest that in vivo methylation of the IAP LTR might be a mechanism contributing to the repression of LTR activity in most organs. This does not exclude the possibility that specific factors are additionally required to promote cell type-specific expression (41), a feature which would account for the relatively small proportion of stained cells in the testis compared with the extent of demethylation of the IAPs, at least at the CpG sites tested, in this organ.

The nonexpression and methylation of the IAP LTR transgene in the somatic tissues is a rather unexpected situation for a promoter which discloses, as observed for many viral sequences as well as for transposable elements such as the human and rat long interspersed nuclear elements (10, 20), the characteristic features (2) of housekeeping CpG island-containing genes: typically, IAP LTRs have a high G+C content (51%; mean genome value, 40%) and lack CpG suppression (CpG/GpC ratio, 1.0; mean genome value, 0.2), they are compact, most of them lack a canonical TATA box, and they possess recognition sites for ubiquitous transcriptional factors (such as Sp1 [22]). Although the observed demethylation in the germ line should clearly be sufficient (and necessary) to pre-

vent heritable CpG suppression in these sequences (see reference 8 for a similar situation), housekeeping genes are generally expressed, and demethylated, in all tissues; this is clearly not the case for the IAPs (or for the long interspersed nuclear elements [44, 54]). This characteristic may represent a specific property of mobile elements and be part of a host strategy to limit the deleterious consequences of their transposition (see below), whereas resemblance to housekeeping genes would be part of a virus strategy for propagation (2).

Biological implications and perspectives. The restricted pattern of expression of the IAP transgenes that we have identified raises interesting questions about the biological relevance of the IAP transcripts previously identified by global Northern blot analyses. IAP transcripts have been detected in several tissues, being rather abundant in the thymus and in early-stage embryos but fairly rare or even undetectable in the testis (reviewed in references 15 and 30). Taking into account that expression of the transgenes has been found to be independent of both the associated reporter gene and the transgene integration sites, and therefore should reflect intrinsic properties of the IAP LTR, a simple explanation for the unexpected occurrence of endogenous IAP transcripts in somatic tissues might be that they originate from a very limited number of IAP sequences which either (i) possess specific and still unidentified regulatory sequences not present in the two IAP LTRs that we have tested, a possibility which cannot formally be excluded (35, 41), or (ii) are simply under the control of adjacent genes or enhancer sequences and therefore subject to position effects (see the Introduction). In accordance with the second alternative, we have recently provided evidence that in the livers of aged mice, >50% of the endogenous IAP transcripts unambiguously originate from a unique IAP sequence (with internal stop codons), in a locus that we have cloned (15, 47). This finding emphasizes that RNA analyses using Northern blotting (or even in situ hybridization) might not be highly significant when one is studying the pattern of expression of highly reiterated elements, because several of them (possibly the nonfunctional ones [see above]) are likely to be activated by nearby genes. Interestingly, a closely related situation also holds for the Ty1 retrotransposon in *Saccharomyces cerevisiae*; one can detect significant levels of Ty1 transcripts which are clearly not productive, as they do not result in transposition, and which most probably originate from the numerous mutated elements that can be detected in the yeast genome (reviewed in reference 3).

At the biological level, it is likely that uncontrolled transposition of IAP sequences should be deleterious for the mouse, and therefore the strong restriction of expression that we have observed might be essential for mouse survival. Yet we have also shown that IAP LTRs can be active in germ line cells, within a narrow time period in the course of spermatogenesis, i.e., in cells in which any transposition-induced mutation will be stably inherited among the following generations. Finally, it appears that IAP transposition is allowed when it might generate genetic diversity, and thus evolution of the species (i.e., in the germ cells), and is repressed when it might be deleterious for the individual (i.e., in somatic cells). This behavior is reminiscent of the germ line-restricted expression of other murine repetitive elements (52) and of the situation in *D. melanogaster*, in which complex regulations take place for several transposable elements (e.g., P-element and I-element regulation of transposition [reviewed in reference 6 and 16]), so that high-frequency transposition is restricted to the germ line and inhibited in somatic tissues. In this respect, the present results possibly reveal an evolutionary conservation in the control of the mobility of transposable elements, from insects to mammals.

Finally, the identification of a biologically relevant niche for IAP activity raises the question of the actual transposition of IAPs in those cells. Interestingly, the transgenic lines that we have developed and which contain the complete IAP-IL3 sequence marked with an intron-containing reporter gene (the transposition of which had been previously demonstrated in tumor cells in culture [23]) could now provide a definite way to demonstrate transposition as well as transcriptional activation of the IAP sequences in those cells. Evidence for transposition would demonstrate activation of both the transgene and the endogenous IAPs, as the latter must necessarily be activated to complement in *trans* the otherwise noncoding IAP-IL3-derived IAP transgene.

ACKNOWLEDGMENTS

We acknowledge P. Briand, D. Bucchini, D. Daegelen, C. Kress, and F. Michiels for help in the introduction of A.D. to transgenesis, D. G. de Rooij and F. Cuzin for many helpful discussions, C. Lavialle and J.-F. Nicolas for critical reading of the manuscript, P. Auriau for animal care, I. Cerutti, A. Loeuillet, P. Medina, and J.-P. Moussu from the Service d'Expérimentation Animale et de Transgénèse (Villejuif, France), where some of the transgenic mice were established, and N. Lenovere.

This work was supported by the CNRS and INSERM and by grants from the ARC (contract 6552), the Ligue Nationale contre le Cancer, and Rhone-Poulenc Rorer (Bioavenir contract).

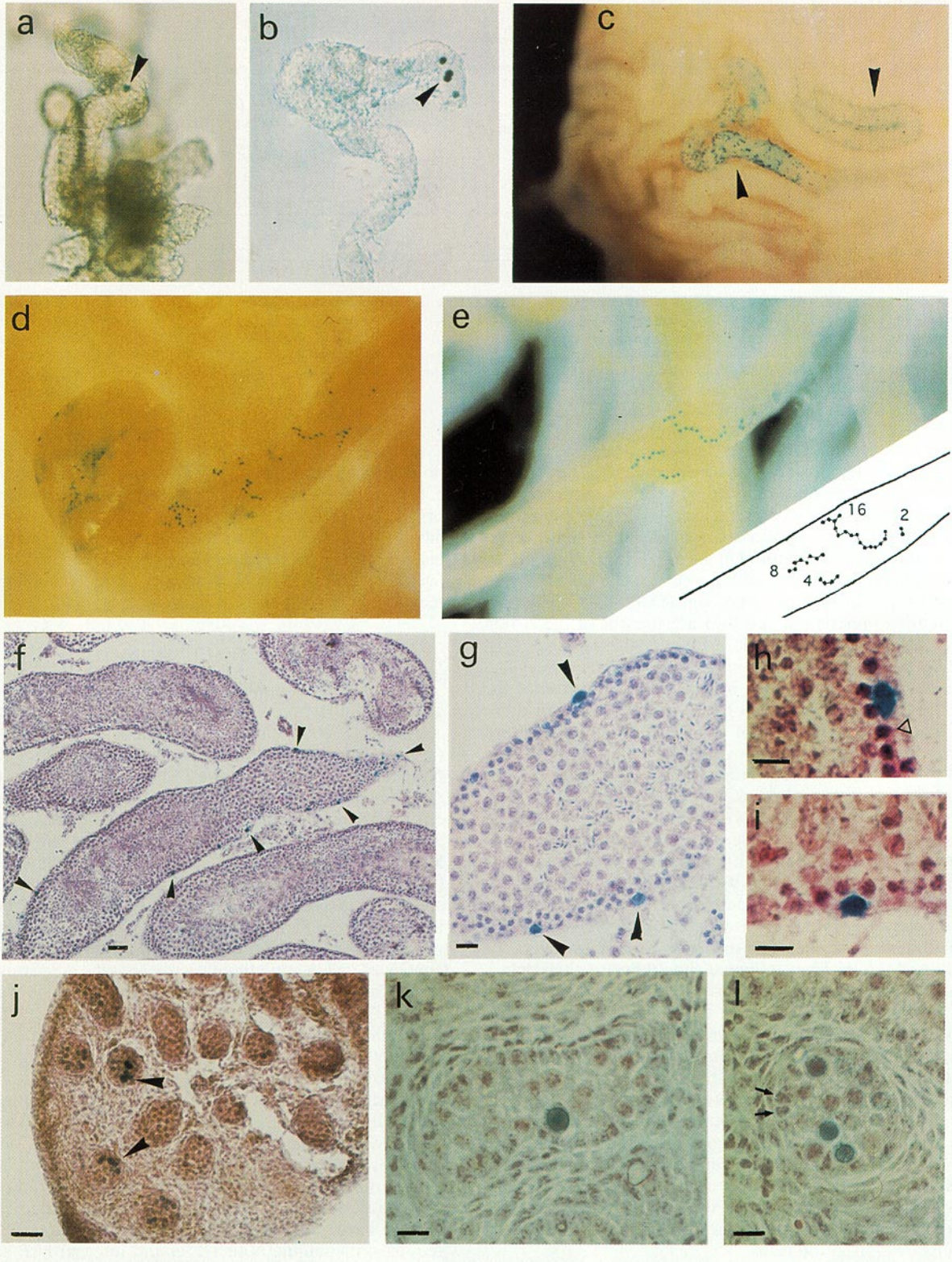
REFERENCES

1. **Bellvé, A. R., J. C. Cavicchia, C. F. Millette, D. A. O'Brien, Y. M. Bhatnagar, and M. Dym.** 1977. Spermatogenic cells of the prepuberal mouse. J. Cell Biol. **74:**68–84.
2. **Bird, A. P.** 1986. CpG-rich islands and the function of DNA methylation. Nature (London) **321:**209–213.
3. **Boeke, J. D.** 1989. Transposable elements in *Saccharomyces cerevisiae*, p. 335–374. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
4. **Bonnerot, C., D. Rocancourt, P. Briand, G. Grimber, and J.-F. Nicolas.** 1987. A β-galactosidase hybrid protein targeted to nuclei as a marker for developmental studies. Proc. Natl. Acad. Sci. USA **84:**6795–6799.
5. **Brinster, R. L., and J. W. Zimmermann.** 1994. Spermatogenesis following male germ-cell transplantation. Proc. Natl. Acad. Sci. USA **91:**11298–11302.
6. **Bucheton, A., C. Vaury, M.-C. Chaboissier, P. Abad, A. Pélisson, and M. Simonelig.** 1993. I elements and the *Drosophila* genome, p. 173–188. *In* J. F. McDonald (ed.), Transposable elements and evolution. Kluwer Academic Publishers, Dordrecht, The Netherlands.
7. **Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter.** 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18:**5294–5298.
8. **Choi, Y.-C., and C.-B. Chae.** 1991. DNA hypomethylation and germ cell-specific expression of testis-specific H2B histone gene. J. Biol. Chem. **266:** 20504–20511.
9. **Clermont, Y., and B. Perey.** 1957. Quantitative study of the cell population of the seminiferous tubules in immature rats. Am. J. Anat. **100:**241–268.
10. **Crowther, P. J., J. P. Doherty, M. E. Linsenmeyer, M. R. Williamson, and D. M. Woodcock.** 1991. Revised genomic consensus for the hypermethylated CpG island region of the human L1 transposon and integration sites of full length L1 elements from recombinant clones made using methylation-tolerant host strains. Nucleic Acids Res. **19:**2395–2401.
11. **de Rooij, D. G.** 1988. Regulation of the proliferation of spermatogonial stem cells. J. Cell Sci. Suppl. **6:**181–194.

11a. **de Rooij, D. G.** Personal communication.

12. **Doerfler, W.** 1991. Patterns of DNA methylation. Evolutionary vestiges of foreign DNA inactivation as a host defense mechanism. Biol. Chem. Hoppe-Seyler **372:**557–564.
13. **Duhl, D. M. J., H. Vrieling, K. A. Miller, G. L. Wolff, and G. S. Barsh.** 1994. Neomorphic agouti mutations in obese yellow mice. Nat. Genet. **8:**59–65.
14. **Dührsen, U., J. Stahl, and N. M. Gough.** 1990. *In vivo* transformation of factor-dependent hemopoietic cells: role of intracisternal A-particle transposition for growth factor gene activation. EMBO J. **9:**1087–1096.
15. **Dupressoir, A., A. Puech, and T. Heidmann.** 1995. IAP retrotransposons in the mouse liver as reporters of ageing. Biochim. Biophys. Acta **1264:**397–402.
16. **Engels, W. R.** 1989. P elements in *Drosophila melanogaster*, p. 437–484. *In*

D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
17. **Falzon, M., J. W. Fewell, and E. L. Kuff.** 1993. EBP-80, a transcription factor closely resembling the human autoantigen Ku, recognizes single- to double-strand transitions in DNA. J. Biol. Chem. **268:**10546–10552.
18. **Falzon, M., and E. L. Kuff.** 1988. Multiple protein-binding sites in an intracisternal A particle long terminal repeat. J. Virol. **62:**4070–4077.
19. **Feenstra, A., J. Fewell, K. K. Lueders, and E. L. Kuff.** 1986. *In vitro* methylation inhibits the promoter activity of a cloned intracisternal A-particle LTR. Nucleic Acids Res. **14:**4343–4352.
20. **Furano, A. V., S. M. Robb, and F. T. Robb.** 1988. The structure of the regulatory region of the rat L1 (L1Rn, long interspersed repeated) DNA family of transposable elements. Nucleic Acids Res. **16:**9215–9231.
21. **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.
22. **Gardiner-Garden, M., and M. Frommer.** 1987. CpG islands in vertebrate genomes. J. Mol. Biol. **196:**261–282.
23. **Heidmann, O., and T. Heidmann.** 1991. Retrotransposition of a mouse IAP sequence tagged with an indicator gene. Cell **64:**159–170.
24. **Heidmann, T., O. Heidmann, and J. F. Nicolas.** 1988. An indicator gene to demonstrate intracellular transposition of defective retroviruses. Proc. Natl. Acad. Sci. USA **85:**2219–2223.
25. **Hogan, B., F. Constantini, and E. Lacy.** 1986. Manipulating the mouse embryo: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **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:**199–204.
27. **Hsiao, W.-L. W., S. Gattoni-Celli, and I. B. Weinstein.** 1986. Effects of 5-azacytidine on expression of endogenous retrovirus-related sequences in C3H 10T1/2 cells. J. Virol. **57:**1119–1126.
28. **Iguchi-Ariga, S. M. M., and W. Schaffner.** 1989. CpG methylation of the cAMP-responsive enhancer/promoter sequence TGACGTCA abolishes specific factor binding as well as transcriptional activation. Genes Dev. **3:**612–619.
29. **Kluin, P. M., and D. G. de Rooij.** 1981. A comparison between the morphology and cell kinetics of gonocytes and adult type undifferentiated spermatogonia in the mouse. Int. J. Androl. **4:**475–493.
30. **Kuff, E. L. and K. K. Lueders.** 1988. The intracisternal A-particle gene family: structure and functional aspects. Adv. Cancer Res. **51:**183–276.
31. **Kuff, E. L.** 1990. Intracisternal A particles in mouse neoplasia. Cancer Cells **2:**398–400.
32. **Kuff, E. L., and J. W. Fewell.** 1985. Intracisternal A-particle gene expression in normal mouse thymus tissue: gene products and strain-related variability. Mol. Cell. Biol. **5:**474–483.
33. **Lathe, R., J. L. Vilotte, and A. J. Clark.** 1987. Plasmid and bacteriophage vectors for excision of intact inserts. Gene **57:**193–201.
34. **Leiter, E. H., J. W. Fewell, and E. L. Kuff.** 1986. Glucose induces intracisternal type A retroviral gene transcription in pancreatic beta cells. J. Exp. Med. **163:**87–100.
35. **Lueders, K. K., J. W. Fewell, V. E. Morozov, and E. L. Kuff.** 1993. Selective expression of intracisternal A-particle genes in established mouse plasmacytomas. Mol. Cell. Biol. **13:**7439–7446.
36. **Lueders, K. K., and J. A. Mietz.** 1986. Structural analysis of type II variants within the mouse intracisternal A-particle sequence family. Nucleic Acids Res. **14:**1495–1510.
37. **McClelland, M., and M. Nelson.** 1992. Effect of site-specific methylation on DNA modification methyltransferases and restriction endonucleases. Nucleic Acids Res. **20:**2145–2157.
38. **McDonald, J. F.** 1993. Evolution and consequences of transposable elements. Curr. Opin. Genet. Dev. **3:**855–864.
39. **McGowan, R., R. Campbell, A. Peterson, and C. Sapienza.** 1989. Cellular mosaicism in the methylation and expression of hemizygous loci in the mouse. Genes Dev. **3:**1669–1676.
40. **Michaud, E. J., M. J. van Vugt, S. J. Bultman, H. O. Sweet, M. T. Davisson, and R. P. Woychik.** 1994. Differential expression of a new dominant agouti allele (Aiapy) is correlated with methylation state and is influenced by parental lineage. Genes Dev. **8:**1463–1472.
41. **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.
42. **Mietz, J. A., 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.
43. **Morgan, R. A., and R. C. C. Huang.** 1984. Correlation of undermethylation of intracisternal A-particle genes with expression in murine plasmacytomas but not in NIH/3T3 embryo fibroblasts. Cancer Res. **44:**5234–5241.
44. **Nur, I., E. Pascale, and A. V. Furano.** 1988. The left end of rat L1 (L1Rn, long interspersed repeated) DNA which is a CpG island can function as a promoter. Nucleic Acids Res. **16:**9233–9251.
45. **Oakberg, E. F.** 1956. A description of spermiogenesis in the mouse and its use in analysis of the cycle of the seminiferous epithelium. Am. J. Anat. **99:**391–413.
46. **Piko, L., M. D. Hammons, and K. D. Taylor.** 1984. Amounts, synthesis, and some properties of intracisternal A particle-related RNA in early mouse embryos. Proc. Natl. Acad. Sci. USA **81:**488–492.
47. **Puech, A., A. Dupressoir, and T. Heidmann.** Unpublished data.
48. **Rugh, R.** 1990. Reproductive systems of adult mice, p. 7–43. *In* R. Rugh (ed.), The mouse: its reproduction and development. Oxford University Press, Oxford.
49. **Sanes, J. R., J. L. R. Rubenstein, and J.-F. Nicolas.** 1986. Use of a recombinant retrovirus to study post-implantation cell lineage in mouse embryos. EMBO J. **5:**3133–3142.
50. **Satyamoorthy, K., K. Park, M. L. Atchison, and C. C. Howe.** 1993. The intracisternal A-particle upstream element interacts with transcription factor YY1 to activate transcription: pleiotropic effects of YY1 on distinct DNA promoter elements. Mol. Cell. Biol. **13:**6621–6628.
51. **Shen-Ong, G. L. C., and M. D. Cole.** 1982. Differing populations of intracisternal A-particle genes in myeloma tumors and mouse subspecies. J. Virol. **42:**411–421.
52. **Trelogan, S. A., and S. L. Martin.** 1995. Tightly regulated, developmentally specific expression of the first open reading frame from LINE-1 during mouse embryogenesis. Proc. Natl. Acad. Sci. USA **92:**1520–1524.
53. **Wivel, N. A., and G. H. Smith.** 1971. Distribution of intracisternal A-particles in a variety of normal and neoplastic mouse tissues. Int. J. Cancer **7:**167–175.
54. **Woodcock, D. M., P. J. Crowther, W. P. Diver, M. Graham, C. Bateman, D. J. Baker, and S. S. Smith.** 1988. RglB facilitated cloning of highly methylated eukaryotic DNA: the human L1 transposon, plant DNA, and DNA methylated in vitro with human methyltransferase. Nucleic Acids Res. **16:**4465–4482.
55. **Ymer, S., W. Q. J. Tucker, H. D. Campbell, and I. G. Young.** 1986. Nucleotide sequence of the intracisternal A-particle genome inserted 5′ to the interleukin-3 gene of the leukemia cell line WEHI-3B. Nucleic Acids Res. **14:**5901–5918.
56. **Yotsuyanagi, Y., and D. Szöllösi.** 1984. Virus-like particles and related expression in mammalian oocytes and preimplantation embryos, p. 218–232. *In* J. van Blerkom and P. M. Motta (ed.), Ultrastructure of reproduction. Martinus Nijhoff, The Hague, The Netherlands.


a
b
c
d
e
16
2
8
4
f
g
h
i
j
k
l