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DETECTION OF IAP RELATED TRANSCRIPTS IN NORMAL AND TRANSFORMED RAT CELLS

I. DJAFFAR[1], L. DIANOUX[1], S. LEIBOVICH[2], L. KAPLAN[3], R. EMANOIL-RAVIER[1] and J. PERIES[1]*

[1] Rétrovirus et Rétrotransposons des Vertébrés - UPR A0043 CNRS
Hôpital Saint-Louis
1 Avenue Claude Vellefaux
75010 - PARIS, FRANCE

[2] URA 1158 - I.G.R - CNRS
39 Rue Camille Desmoulins
94805 - VILLEJUIF CEDEX, FRANCE

[3] U.149 INSERM
123 Boulevard de Port-Royal
75014 - PARIS, FRANCE

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Intracisternal A-particles (IAP) genes in variable copy number exist in all rodent species studied. Expression is highly repressed in murine normal cells except in embryonic and transformed cells. We searched for IAP related sequences expression in another rodent species cells. Using the more conserved sequence of IAP gene between mouse, Syrian hamster, and rat species (0.4 kb HindIII-PstI fragment from $PMIA_{14}$), we have been able to detect IAP related transcripts in rat cells. We found that, i) IAP related transcripts are poorly expressed in normal cells, since among 10 tissues tested, only the placenta shows IAP RNA. ii) IAPs are highly expressed in all the transformed cells tested. iii) the detected transcripts appear to have similar sizes in rat cells as in mouse cells (7.2 kb ; 5.4 kb). None of the probes corresponding to other regions of the IAP gene nor the entire sequence of PMIA14 allowed us to detect such transcripts.

Murine intracisternal type A particles (IAPs) are defective retrovirions encoded by members of a large family of endogenous proviral elements (500 to 1000 per haploid genome) (1-5).

Sequences homologous to the mouse IAPs have been detected as repetitive families in the genome of rodents and possibly other mammals (6). They are particularly numerous in syrian hamster (700 to 950) (7) and in rat (500 copies) (8)

IAPs are generally considered to be unassociated with any type of pathogenicity (9-11). The IAP genes family have been studied as a model system for gene regulation and genome plasticity via retrotransposition of

* Corresponding author.

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proviral insertions. This phenomenon occurs by reverse transcription of retroviral sequences in cells containing type-A particles (12-16). The effects of IAP proviral insertions include positive gene regulation, amplifications of IAP elements, and reverse transcriptase and integrase formation as protein products, which may be at the origin of other retrotransposition events. Even if it is difficult to elucidate a true role for IAP during cell proliferation or malignant transformation, it becomes clear that the genetic instability during tumor cell population development may be influenced by IAP sequences particularly in the clonal selection phase of the malignant process.

The IAP sequences are abundantly expressed in murine tumor cells as well as in germ line but are not naturally expressed in other species. IAP particles have been reported in rat tumor derived cell lines (17-19) but morphological evidence of the presence of such particles is not conclusive. In fact, no RNA expression of IAP related sequences in other species than mouse and Syrian hamster has been evidenced. In order to detect an RNA expression of IAP related sequences in any species we have used as probe a small fragment of murine IAP gene highly homologous to syrian hamster IAP.

We report here the way used for detecting IAP related RNA sequences expression in different rat cell lines or tissue types. For the first time, we were able to detect IAP expression in rat cells and placenta. Detection of IAP related transcripts was done by Northern blot under stringent conditions of hybridization.

MATERIALS AND METHODS

Cells and tissues

SDV_1 and F_{11} cell lines were kindly provided by Guillouzo (Rennes, France) and were grown in Williams E Medium (Eurobio, Paris). PMC9 and ERD were kindly provided by Evrard (Paris, France) and were grown in DMEM F12 medium (Eurobio, Paris). KiNRK and XC cells have been obtained from American Type Culture Collection. Rat myogenic cells (22) were grown in DMEM H16 (Eurobio, Paris). Rat tissues (placenta, brain, liver, lung, kidney, ovary, testis, spleen, thymus, embryonic muscle) were quickly frozen in liquid nitrogen after harvesting.

Electron microscopy

Cells were examined with a "Philips 301" electron microscope. They were fixed in 2.5 % glutaraldehyde, post-fixed in 1 % osmic acid and embedded in epon before staining with uranyl acetate and lead citrate. At least, 250 sections were systematically scanned for each cell sample. Care was taken to avoid observed identical or proximal fields. Samples were coded prior to examination to eliminate prejudice. Morphology, size and localisation requirements of IAP are as follows : intracisternal location,maturation by budding from the reticulum membrane, diameter of 80-90 nm, two electron dense rings marged with the inner lamina of the unit membrane and no intermediate layer between the nucleoid and the external envelop.

RNA isolation and blot hybridization

Total cellular RNA from all the cell lines listed in table I as well as Ki-Balb cells was isolated by lysis of exponentially growing cells in Urea-LiCl according to Auffray and Rougeon (23). 50 ug of each RNA sample were denatured by glyoxal, subjected to electrophoresis in 1,1 % agarose gel and transferred to nitrocellulose as previously described (24) except that baked filters were treated in boiling Tris 20mM pH8 for 10 mn. Filters were then prehybridized and hybridized in 50 % formamide at 42°C (24). Filters were washed under stringent conditions : 2x5 mn in 2xSSC, 0.1 % SDS at 25°C and 2x20 mn in 0.1xSSC, 0.1 % SDS at 50°C.

Total cellular RNA from all the tissues used as well as L6 myoblasts was isolated by the guanidium thiocyanate procedure (25). The poly(A) containing fraction of the RNA from L6 myoblasts was selected by retention on oligo(dT) cellulose under previously specified conditions (26). Gel electrophoresis, Northern blot analysis and filter washing were done as indicated above.

Molecular probes

The PMIA14 clone was kindly provided by Heidman (Villejuif, France). It represents the entire IAP genome (2). The 0.4 kb Hind III/PstI as well as the 0.3 kb PvuII/EcoRI were obtained by Hind III + PstI or PvuII + EcoRI digestion of PMIA14. The pAM91 actin cDNA was cleaved with PstI to isolate a 1150 bp insert containing sequences common to all actin genes. The 1.3 kb PvuII/PvuII was obtained by PvuII digestion, 1.5 kb PstI/PstI by PstI digestion and 1.4 kb Bam HI/Bam HI, by Bam HI digestion of PMIA14. The products of digestion were migrated in a 8 % acrylamide gel, and the fragments were eluted in 0.5M ammonium acetate, 1mM EDTA pH8. The fragments were then labelled by random priming with a commercial kit (Boehringer, Mannheim) to achieve specific activities of about 1.10^9 cpm/ug.

RESULTS

Heteroduplexes between mouse and rat clone analyses have shown that rat IAP sequences are composed of short regions of homology interspersed with regions of non homology. Furthermore, divergence of IAP sequences is greater between rat family members(as indicated by the fact that the Tm of the hybrid formed between a rat probe and rat genome DNA is 6°C below the Tm of the self hybrid) than between mouse or syrian hamster family members (respectively, only 1°C and 1.5°C below the Tm of the self hybrids) (2). Similarly low Tm's were found for hybrids between the rat probe and several other cloned rat IAP sequences. The different subsets of sequences are not known in their length and in their boundaries with LTR.

Nevertheless, some short regions of homology exist between the different rat IAP clones isolated, or between mouse IAP and rat IAP, and, thus, represent potential probes for detecting IAP related sequences in any species cells. We have used such probes to detect RNA expression of IAP related sequences in rat cells.

In figure 1, a physical map of $PMIA_{14}$ (2) is shown. This clone is colinear to the 35S IAP RNA and thus, represents the entire IAP genome. By heteroduplex analysis, Lueders and Kuff have shown that there were some regions homologous between mouse, syrian hamster and rat sequences and

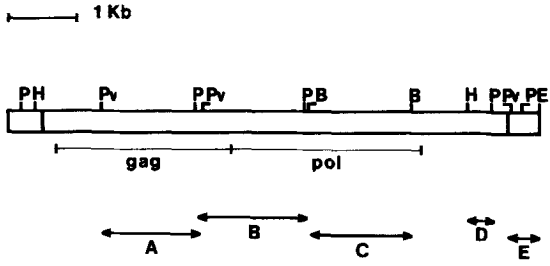

Figure 1

Physical map of mouse pMIA14.

Partial restriction of PMIA14 containing the 7.2 kb IAP. The ends of the gene are delimited by long terminal repeats (LTRs :). Fragments used as mouse IAP probes :

A : 1.3 kb PvuII/PvuII
B : 1.5 kb PstI/PstI
C : 1.4 kb Bam HI/Bam HI
D : 0.4 kb HindIII/PstI
E : 0.3 kb PvuII/EcoRI

Restriction sites are as follow : P, PstI; H, HindIII; Pv, PvuII; B, BamHI; E, EcoRI.

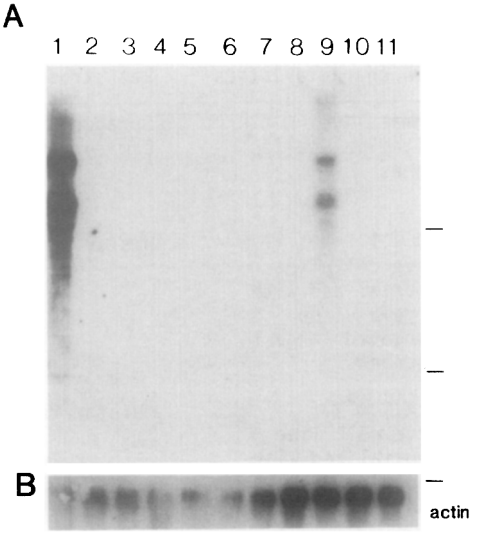

Figure 2

Analysis of IAP related transcripts in normal rat cells by Northern blotting.

Total cellular RNA was isolated by the guanidium thiocyanate procedure. 25 ug of each RNA sample was denatured by glyoxal, subjected to electrophoresis in 1,1% agarose gel, transfered to nitrocellulose and hybridized with A : the 0.4 kb HindIII/PstI fragment from PMIA14; B : the pAM91 actin cDNA. Filters were washed under stringent conditions : 2x5 mn in 2xSSC, 0.1% SDS at 25°C and 2x20mn in 0.1xSSC, 0.1%SDS at 50°C.

RNA sources were : lane 1 : Ki-Balb, lane 2 : Brain, lane 3 : liver, lane 4 : M.E., lane 5 : lung, lane 6 : kidney, lane 7 : ovary, lane 8 : testis, lane 9 : placenta, lane 10 : spleen, lane 11 : thymus.

28S and 18S ribosomal RNA markers are indicated by small dashes.

determined that the highly conserved sequences were in the env region retrovirus-like elements in these 3 species, as well as in all clones tested from one species (8). Because of relatively low overall sequence homology, the mouse IAP full length probe cross hybridize with the rat IAP mRNA only in low stringency conditions. However, in low stringency conditions, the IAP probe could cross-hybridize with highly conserved Pol-containing non-IAP sequences, leading to high background. Therefore, we designed as a probe a fragment of the "env" region of mouse IAP (0.4 Kb Hind III/Pst I fragment of PMIA14), corresponding to a sequence highly conserved between rat and mouse IAP genes. This sequence did not contain the pol region and thus could be used under high stringency conditions. None of the probes corresponding to other regions of the mouse IAP gene (fragments A, B, C, E indicated in figure 1), nor the entire IAP gene (PMIA14 probe) allowed us to detect such transcripts. Since this 0.4 Kb Hind III/Pst I from $PMIA_{14}$ could be included in this highly conserved region it was used in the following experiments.

Table 1

List of rat cell lines used

Designation	Main characteristics	Rat strain	References
SDV1 F11	Normal liver epithelial cells clones	Sprague-Dawley rat	Gift of Guillouzo, Rennes, France
PMC9	M.C.R.1.1 Nerve cell line Immortalized with polyoma virus truncated large T sequences	Wistar rat	Gift of Evrard, Paris France
ERD	E1.A.R.4.1. nerve cell line immortalized with adenovirus E1A sequences	Wistar rat	Gift of Evrard, Paris, France
KINRK	Kirsten MSV transformed rat kidney cells		De Larco, NCI, NIH, Bethesda ATCC CRL 1569
XC	RSV-induced tumor derived cell line		ATCC CCL 165
L6FBJ2	Myoblastic v-fos-transfected cells		Leibovitch Villejuif, France

First we searched detection of IAP related transcripts in normal rat cells. Total RNA prepared from tissues was analysed on Northern blots (figure 2). The only detectable signal was obtained with placenta sample. We found 2 transcripts (7.2 kb and 5.4 kb), similar to the major IAP-related transcripts described in the mouse. All other tissues gave negative results. This indicates that IAP related RNAs are not expressed in most normal tissues. It is important to note that we were unable to detect any IAP RNA transcripts in the rat thymus, where the highest level of IAP RNA is detected in the mouse (27).

Since mouse IAP are frequently expressed in transformed cells, we wondered if it was true for rat cells. Thus, we tested IAP expression in 2 different rat cells cultured in vitro.

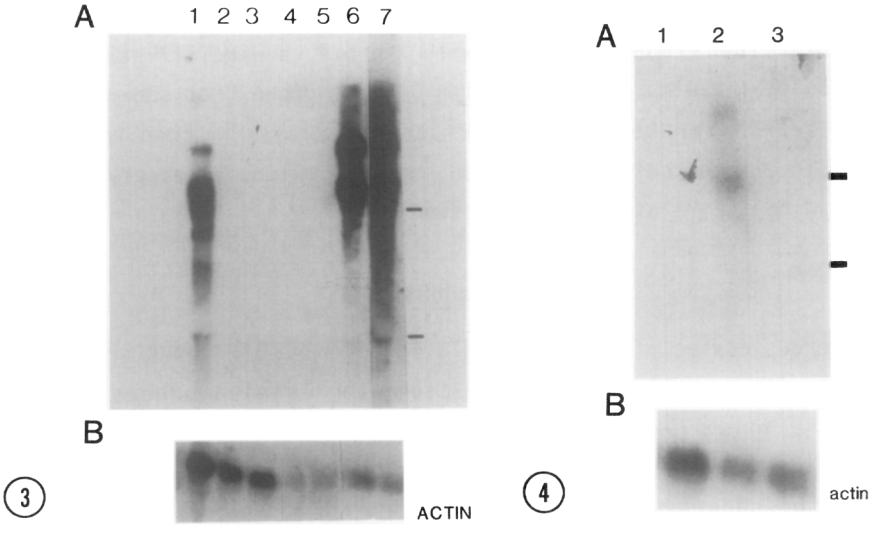

Figure 3

Northern blot analysis of IAP related transcripts.

Total cellular RNA was isolated by lysis of exponentially growing cells in LiCl-Urea. 50 ug of each RNA sample or 10 ug of Ki-Balb were electrophoresed, bloted and hybridized to ^{32}P labeled probe as described in figure 2. Sources of RNA were as follows : lane 1 : Ki-Balb, lane 2 : ERD, lane 3 : F11, lane 4 : PMC9, lane 5, SDV1, lane 6 : Ki NRK, lane 7 : XC. The position of migration and sizes of 28S and 18S RNA markers are indicated. A : Hybridization with the pMIA14 fragment; B : Hybridization with the pAM91 actin probe.

Figure 4

Northern blot analysis of IAP related transcripts in L6 myoblasts, compared with v-fos-induced transformants.

Total cellular RNA was isolated by the guanidium thiocyanate procedure, and selected for poly(A) containing sequences by retention on oligo(dT) cellulose. Aliquots of 5 ug of poly(A) containing RNA were processed for gel electrophoresis and Northern blot hybridization. Sources of RNA : lane 1 : L6 cells, lane 2 : clone LA, lane 3 : clone LT3. Sizes of 28S and 18S RNA markers are indicated. A : Hybridization with the pMIA14 fragment; B : Hybridization with the pAM91 actin probe.

In the first system, we used the 6 different rat cell lines listed in table 1. They originated from various tissues of several rat strains and are either virally or chemicaly transformed or immortalized or normal cells.

Total RNA (50 ug per lane) was loaded on an agarose gel and subjected to Northern blot analysis. As shown in figure 3, IAP related RNAs were only detectable in XC and Ki-NRK transformed cells. Even after a longer exposure of the blot, no transcripts were found in normal (SDV1, F11) or immortalized cells (PMC9, ERD) (data not shown).

In the second system, we used a clonal line of rat myogenic cells transfected with v-fos which had previously been studied by one of us (22). Results presented in figure 4 show that an IAP related expression can be detected in transformed myogenic cells which have integrated an entire copy of v-fos (clone LA). On the contrary, a cell line which has a deleted copy of v-fos (clone LT3) behaves like the control cell line, and shows no IAP RNA.

Same data were obtained by rehybridization of the same blots with a plasmid containing the rat IAP specific insert (kindly provided by Kuff, NIH, Bethesda) : the signals obtained with the 0.4 kb HindIII/PstI fragment from $PMIA_{14}$ comigrated with those seen with IAP rat probe (not shown).

The search for retroviral IAP particles by electron microscopy was negative in all the cell lines tested, despite extensive observation, even in those showing IAP related RNA expression.

DISCUSSION

Numerous copies of sequences related to mouse IAP genes are found in the genome of other species by hybridization with labeled probes representing the entire mouse 7-kb IAP element (5). These persistent signals of rat DNA after stringent washings confirm the presence of conserved and/or repetitive IAP-related sequences. Although IAP related genes are present in the genome of many species the natural expression of viral particles has only been reported in the Syrian hamster species (7). Since mouse IAP genes might be implicated in embryogenesis and tumor progression (20), the understanding of their true role could benefit from the finding of expression of IAP related genes in other species. In this study, we report a way to detect IAP related transcripts in cells of another species, and checked it on rat species. About 500 IAP-related sequences were estimated to be present in the rat genome, and their main characteristics are their heterogeneity and their divergence from one another (16). Particles have been described in some rat transformed cells (28-32) but it is far from clear whether they are really IAP.

The choice of the probe was primordial to obtain this signal. Because of relatively low overall sequence homology between IAPs of all species, the

mouse IAP full length probe cross hybridizes with the rat IAP mRNA only in low stringency conditions. However, in low stringency conditions, the IAP probe could cross-hybridize with highly conserved Pol-containing non-IAP sequences, leading to high background. Therefore, we designed as a probe a fragment of the "env" region of mouse IAP (0.4 kb HindIII/PstI fragment of PMIA14), corresponding to a sequence highly conserved between IAP genes of 3 species (mouse, rat, Syrian Hamster) (8). This sequence did not contain the pol region and thus could be used under high stringency conditions. Using this probe, we were able to detect IAP RNA in rat cells. None of the probes corresponding to other regions of the mouse IAP gene (fragments A, B, C, E indicated in figure 1), nor the entire IAP gene (PMIA14 probe) allowed us to detect such transcripts. Using a cloned rat IAP probe, we checked that those rat RNA transcripts were true and migrating at the same size.

Our findings in rat species are in good agreement with the observations obtained with mouse species : i) IAP related transcripts are poorly expressed in normal cells; among 10 tissues tested, only the placenta shows IAP RNA. Thus, these results do not exclude, of course, the existence of a lower expression in other tissues, undetectable by Northern blot analysis. ii) IAP are highly expressed in all the transformed cells tested. Thus, as in the mouse, IAP transcripts seem to be more frequently expressed in transformed cells than in normal ones. iii) The detected transcripts in rat cells appear to have similar sizes as in mouse (7.2 kb; 5.4 kb) (33). However during the progression of this work, we tested a cell line established from a Wistar rat pleural mesothelial tumor induced by Chrisotyle (34). The transcripts detected in these cells were smaller (4 kb;4.5 kb) and were undetectable in the normal cell (not shown). This could be explained by an expression of deleted genomic elements, as previously described in murine myeloma (20). In our case, these smaller transcripts may reflect the heterogeneity of IAP related sequences in the rat genome.

Northern-blot analysis supports our finding that IAP-homologous sequences are expressed in rat placenta. The placenta, an organ of embryonic origin, provides hormones, growth factors and effectors of cell differentiation during the development of the foetus which involve to switch on and switch off many different genes in a programmed way. Between various mechanisms proposed to explain the hypothetical role of viral endogenous sequences, the reverse transcriptase (RT) may play a crucial role via retrotransposition events. Expression of retrovirus-like sequences (RTVL-H) was found in human placenta (34) and are good candidates for retroposons. It is very likely that RT is involved in these successive gene activations-inhibitions and that placenta may be an excellent tissue for studying them.

Our results concerning RNA expression in rat myogenic cells transfected with v-fos raise some questions. Transcription of IAP -related sequences was detected in rat myogenic cells transfected with entire v-fos genome but not with deleted v-fos genome. It is likely that integration pattern of v-fos in cellular genome is not the origin of these expression. Only the entire v-fos genome-transfected cells were transformed cells. The possibility of linkage between oncogene expression and IAP transcription might exist even if the studies are still preliminary. IAP genes expression might be mediated by the enhancing action of viral or cellular oncogenes on the promoter activity of IAP LTRs. The promoter activity might be secondary to a cellular transformation process, if we consider that IAP elements do not have any programmed role in cell transformation.

The expression features of rat IAP related transcripts (i.e. poorly expressed in normal tissues, often expressed in transformed cells) are strongly reminiscent of those of mouse IAPs. The discovering of regulated expression of IAP related genes in different species-specific cells could help to define a true role for these genes.

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