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Comparison of the sequence organization of related retrovirus-like multigene families in three evolutionarily distant rodent genomes

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ABSTRACT

Sequences related to mouse intracisternal A-particle (IAP) genes have been isolated from rat and Syrian hamster gene libraries as recombinants in λ phage. The sequences are moderately reiterated in both these genomes but their sequence organization in the hamster genome is different from that in the rat genome. Restriction analysis and electron microscopy indicate that the Syrian hamster IAP sequences represent a family of relatively homogeneous well-conserved units; in this, they resemble the mouse IAP genes. The rat sequences, in contrast, are heterogeneous. Both the hamster and rat IAP sequences contain regions homologous to mouse IAP genes interspersed with regions of apparent non-homology. The interspersed regions range in size from 0.5-1.0 kilobases (Kb). The regions of homology among the mouse, rat and Syrian hamster IAP sequences have been mapped to a 5-6 Kb internal region on the mouse IAP genes. Mouse IAP long terminal repeat (LTR) sequences were not detected in the rat and Syrian hamster genomes.

We used the thermal stability of hybrids between cloned and genomic IAP sequences to measure family homogeneity. Mouse and Syrian hamster IAP sequences are homogeneous by this criterion, but the rat IAP sequences are heterogeneous with a T_m 6°C below the self-hybrid. The contrasting organization of IAP-related elements in the genomes of these rodents indicates that amplification or homogenization of this sequence family has occurred independently and at different periods of time during their evolution.

INTRODUCTION

IAP genes occur in the mouse genome as dispersed homologous but non-identical 7 Kb units; a number of these have been isolated from libraries of mouse DNA in λ phage and have been characterized in considerable detail (1-3). In a previous study, cloned mouse IAP genes were used as probes to identify related sequences in the DNAs from a variety of animal cells (4). DNAs from rat, gerbil, and hamster cells all showed strong hybridization. The pattern and intensity of hybridization to genomic blots suggested that the sequences were reiterated in these species as they are in the mouse. We have now used mouse IAP gene probes to isolate related sequences from libraries of rat and Syrian hamster DNAs in λ phage and characterized the organization of these sequences in the respective genomes. Suzuki et al. (5) have also recently

reported cloning IAP sequences from the Syrian hamster genome.

METHODS

Sources of DNA

High molecular weight DNA was prepared as previously described (1) from the livers of Syrian hamsters (Mesocricetus auratus), various strains of rats (Rattus norwegicus) obtained from the NIH Small Animal Section, and Chinese hamsters (Cricetulus griseus) obtained from Cambridge Diagnostics, Cambridge, MA. Rattus rattus liver DNA was provided by Anthony Furano, NIH. The rat genomic library (6) was provided by Thomas Sargent, NCI, and the Syrian hamster genomic DNA library by Brian Crawford, Los Alamos National Laboratory. The recombinant mouse IAP clones λMIA2, λMIA14, λMIA58, and pMIA1 have been described (1). All recombinant genomic DNA libraries were in Charon 4A phage.

Miscellaneous Procedures

Subcloning of DNA fragments (1), restriction enzyme digestion and agarose gel fractionation of DNAs (1), nitrocellulose filter hybridization (1), radioactive labeling of probes by nick-translation (7), and heteroduplex analysis (8) have been described. Blot hybridization with heterologous probes was carried out in 6 x SSC at 50°C followed by washing in 6 x SSC at 55°C (1 x SSC = 0.15 M NaCl, 0.015 M Na Citrate, pH 6.4); hybridization with homologous probes was at 65°C, followed by washing in 0.1 x SSC at 55°C.

Thermal Stability Determination

Radioactive probes were hybridized with 80 μg of genomic DNA (C_0t=240), 2 μg of plasmid DNA (C_0t=6) or 4 μg of phage DNA (C_0t=12). C_0ts are calculated on the basis of total driver DNA concentration. Incubations were generally one hour (at 65°C for homologous sequences and 50°C for heterologous sequences). DNAs used to drive the reactions were sheared by sonication: genomic DNAs were sonicated to an average size of 450 bp as previously described (9); supercoiled plasmid DNAs were heated in 0.1 x SSC for 10 min. in a boiling water bath before sonication in the presence of E. coli DNA; phage DNAs were sonicated in the same way without the boiling step. The incubated samples were diluted 20-fold with 0.12 M sodium phosphate (1:1) and loaded on hydroxylapatite columns in the same buffer at 45°C. Three 1 ml fractions were eluted at each 5°C increment up to 95°C.

RESULTS

Isolation of IAP-Related Sequences from Rat and Syrian Hamster Genomic Libraries

Recombinants containing sequences related to mouse IAP genes were selected from rat and Syrian hamster genomic DNA libraries using a cloned mouse IAP gene fragment as probe. Hybridization was carried out under conditions designed to permit association of divergent sequences. Six isolates from each library were studied; clones selected from the rat library were designated λRIAs and those from the Syrian hamster library λSHIAs.

The reiteration frequency of the IAP-related sequences in the genomes of these species was estimated from the fraction of plaques in the genomic libraries which reacted with a cloned mouse IAP gene fragment. These were as follows: rat 0.5%, Syrian hamster 0.8%, and mouse 1.1%. Using the previously determined value of 1000 copies of IAP genes for the mouse genome (9), the number of copies in the Syrian hamster genome appears to be in a similar range. A reiteration frequency of 950 copies was calculated by Suzuki et al. (5). The number of copies in the rat genome appears to be about half of that in the mouse.

Determination of Regions of Homology Between Mouse, Rat and Hamster IAP Sequences by Heteroduplex Analysis

Representative heteroduplexes formed with recombinants containing the 7 Kb mouse A-particle genes and two isolates from the rat gene library are shown in Figure 1. Figure 3A shows a similar pairing of a mouse IAP gene and Syrian hamster IAP sequences. There were short regions of homology interspersed with regions of non-homology in the areas corresponding to the mouse IAP gene. The homology regions appeared to have a range of thermal stabilities because some homologies were always seen while others were present only in some heteroduplexes (compare the two molecules in Figure 1B).

The regions of homology between the mouse and rat or mouse and Syrian hamster sequences were generally entirely within the mouse IAP genes, between map positions 0.5-6.0 Kb (see Figure 4) and were ordered in a linear fashion with respect to the mouse IAP genes. Homology between the hamster and mouse IAP sequences extended closer to the ends of the mouse genes than that with the rat IAP sequences. We did not observe homology between the IAP-related elements isolated from rat and Syrian hamster and the LTR regions of the mouse genes. The interspersed regions of non-homology ranged from 0.5-1.0 Kb and in most pairings, the two single-stranded arms were similar in size. This suggests that the non-homologous regions have arisen from divergence rather

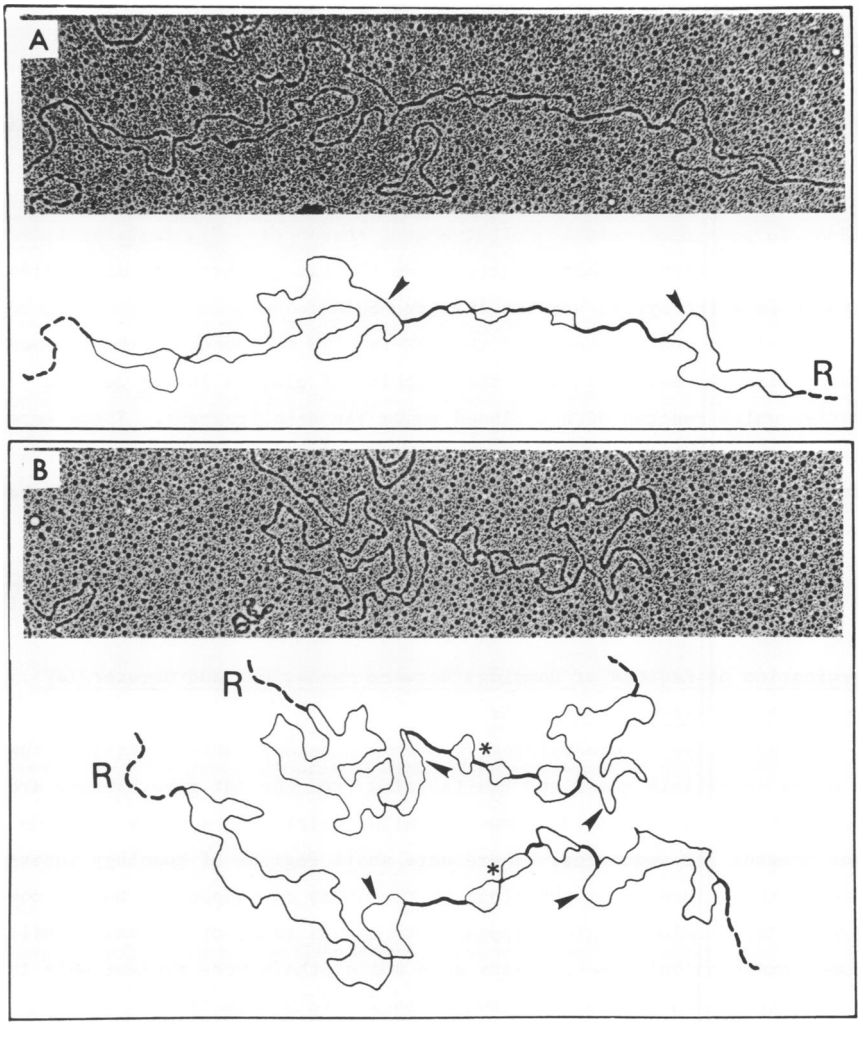

Figure 1. Determination of homologies between rat and mouse IAP sequences by heteroduplex analysis. SV40 DNA (5.24 Kb) and Øx174 DNA (5.386 Kb) were used as double and single stranded standards, respectively in this figure as well as Figures 2 and 3. A. Heteroduplex formed between λRIA3 and λMIA2. B. Heteroduplex formed between λRIA10 and λMIA14. The bottom drawing includes an additional heteroduplex between this pair to show the variation in stability of the homologous regions; the region to note is indicated with an asterisk. In the drawings in this Figure as well as Figures 2 and 3, heavy lines indicate regions of homology, thin lines regions of nonhomology, and broken lines λ arms; the short λ arm is marked R. Arrows delimit the 7 Kb mouse IAP genes in each heteroduplex.

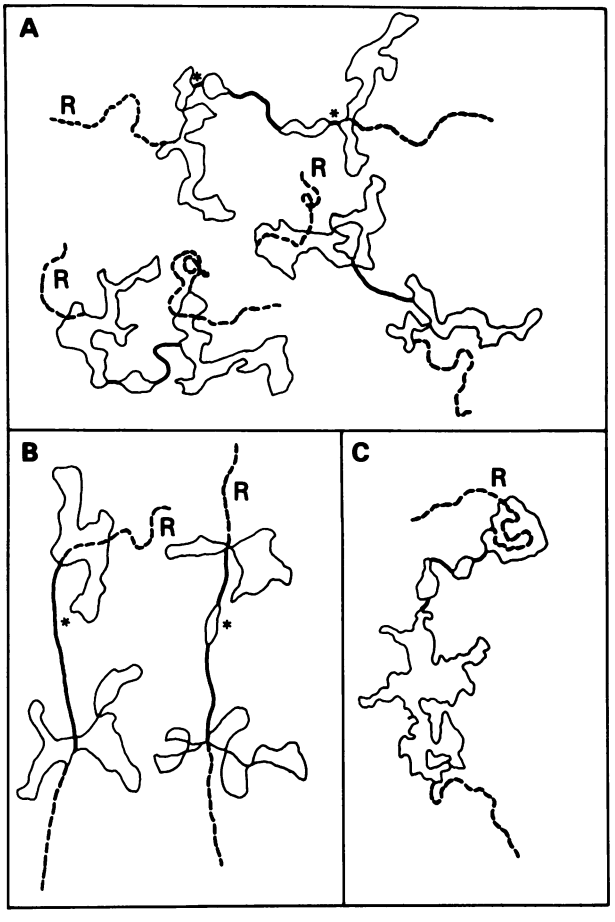

Figure 2. Schematic diagrams of heteroduplexes formed between cloned rat IAP sequence elements. A. Heteroduplexes formed between λRIA3 and λRIA106; B. λRIA105 and λRIA106; C. λRIA3 and λRIA105. Regions showing variation in stability are indicated by asterisks.

than insertions and deletions and that conservation of the linear order and length is important. Figure 4 summarizes the extent and location of regions of homology between the various clones derived from heteroduplex analysis with reference to the mouse IAP genes.

Determination of Sequence Organization in the Rat and Syrian Hamster IAP Units by Heteroduplex Analysis

Heteroduplexes between a pair of Syrian hamster IAP clones (Fig. 3B) showed continuous homology for 7-8 Kb. Heteroduplexes between pairs of rat

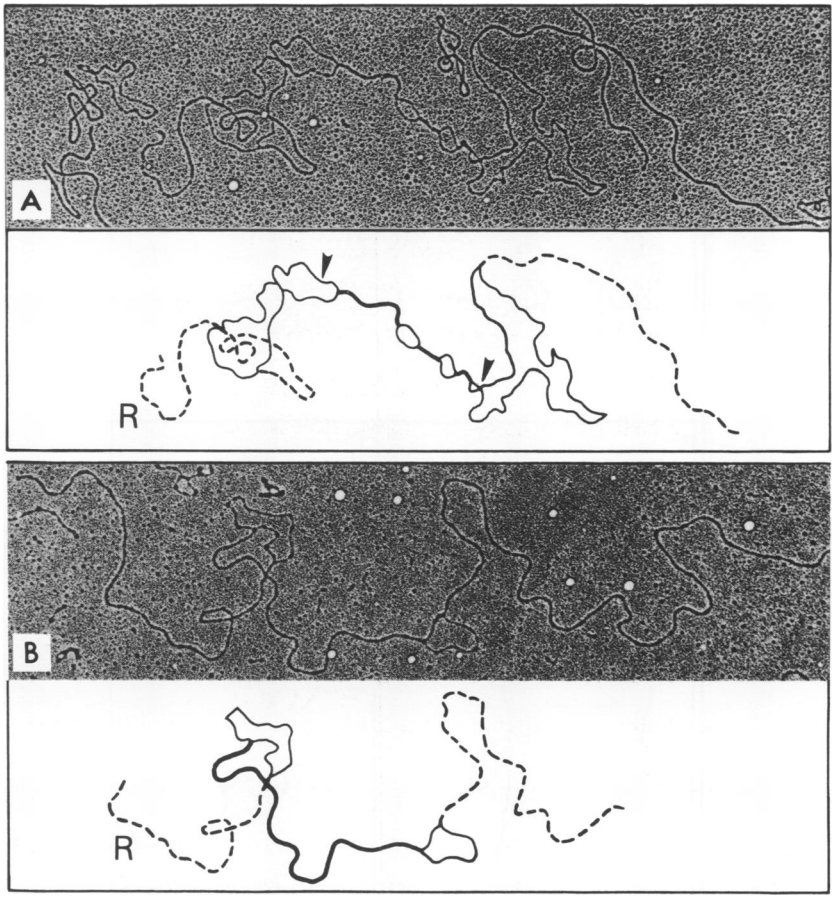

Figure 3. Heteroduplex analysis of Syrian hamster IAP sequences. A. Heteroduplex formed between λSHIA12 and λMIA2. B. Heteroduplex formed between λSHIA12 and λSHIA1.

IAP clones gave an entirely different result. None of the combinations formed stable regions of continuous homology. Instead, pairings between individual rat IAP sequences (Fig. 2) looked very much like those between rat and mouse sequences (see Fig. 1), with short regions of homology interspersed with regions of non-homology. Even homologous regions had variable degrees of stability as shown for three heteroduplexes formed between λRIA3 and λRIA106 in Figure 2A and for λRIA10 and λRIA106 in Figure 2B. The sizes of the non-homologous regions were again 0.5-1 Kb and were similar in size in both partners in the heteroduplexes.

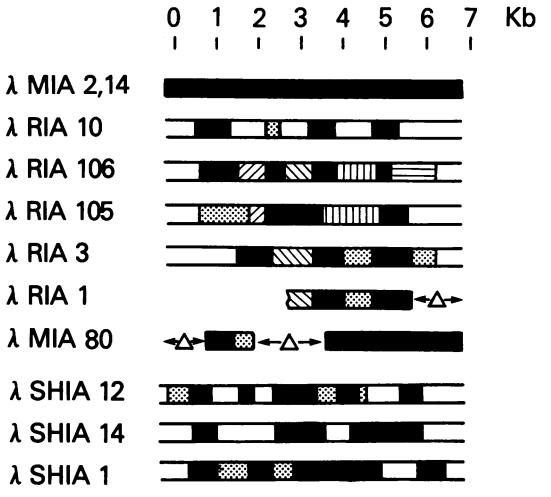

Figure 4. Extents of homologies between rat, Syrian hamster, and mouse IAP sequences. Regions of homology of the various rat and hamster IAP sequences determined by measuring heteroduplexes are shown relative to the physical map of mouse IAP genes (1,2). Regions of strong homology with mouse genes are indicated by filled bars, regions of weaker homology are indicated by stippled bars, and regions of non-homology by open or line pattern bars. Homologies between individual rat genes in regions that are not homologous to mouse sequences are indicated by various line patterns. Deletions and their extent are indicated by open triangles flanked by arrows. λMIA80 is a previously undescribed mouse IAP sequence containing two deletions as well as a 0.5 Kb insertion with respect to the full size gene.

There were homologies between the rat sequences in regions which had no apparent homology with the mouse IAP genes (λRIA3 and λRIA106, Fig. 2A). Other regions seemed to be present only in some of the rat clones. For example a region of λRIA106, that lacked homology with mouse IAP genes, shared homology with λRIA105 but not with λRIA3. Thus, the rat IAP sequences appear to be heterogeneous units composed of several subsets of sequences, only some of which are related to mouse IAP genes.

Organization of the Syrian Hamster and Rat IAP Sequences from Restriction Analysis

We selected for further study a hamster clone (λSHIA12) and a rat clone (λRIA3) each of which showed extensive homology with the mouse IAP genes. Regions of the phage DNAs that were homologous to the mouse plasmid clone pMIA1 (Fig. 5) were subcloned into plasmids for use as probes and for restriction analysis. Two PstI fragments, 3.6 Kb and 3.4 Kb in length, respectively, were subcloned from the Syrian hamster recombinant. From the rat recombinant

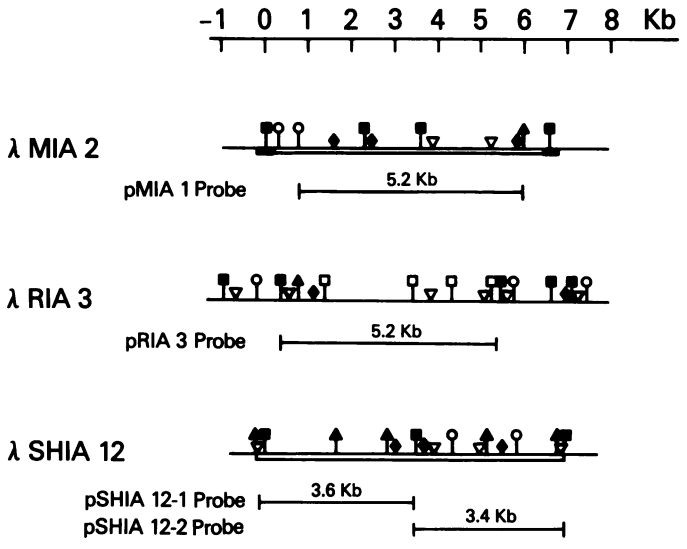

Figure 5. Physical maps of mouse, rat and Syrian hamster IAP clones. Top. Partial restriction map of recombinant λMIA2 containing the 7 Kb mouse IAP gene(double line); the IAP gene (double lines) is delimited by long terminal repeats (LTRs) indicated by solid bars. Mouse flanking sequences are shown as a single line. A 5.2 Kb EcoRI/HindIII fragment from a pBR322 recombinant designated pMIA1 (1), was used as mouse IAP probe. Center. Restriction map for the IAP-related sequences in the rat recombinant λRIA3. The ends of the unit have not yet been defined. A 6 Kb EcoRI fragment from this recombinant was subcloned into pBR322 as pRIA3. A 5.2 Kb PstI fragment from this plasmid was used as probe for rat IAP sequences. Bottom. Restriction map for the IAP-related sequences in the Syrian hamster recombinant λSHIA12. The 3.6 and 3.4 Kb PstI fragments subcloned into pBR322 are designated pSHIA12-1 and pSHIA12-2, respectively and were used as Syrian hamster IAP sequence probes. Restriction sites are: , PstI; , EcoRI; , PvuII; , BamHI; , HindIII; , BglII.

we subcloned a single 6 Kb EcoRI fragment, from which a 5.2 Kb PstI fragment was prepared for use as probe. The restriction maps for the mouse, Syrian hamster and rat IAP sequences are shown in Figure 5.

Comparison of the restriction patterns of the isolated genes with those of the respective genomic DNAs revealed striking differences between the organization of IAP sequences in the Syrian hamster and rat genomes. Hybridization of labeled Syrian hamster IAP probes to λSHIA12 and to hamster genomic DNA is shown in Figure 6, lanes 1-4. When genomic DNA was cut with enzymes such as BamHI (or PstI, not shown), which cut entirely within the IAP-related sequence in λ-SHIA12, discrete reactive fragments (3.9, 1.6 and 1.3 Kb) were produced which were identical in size to those seen with the isolated gene. It is

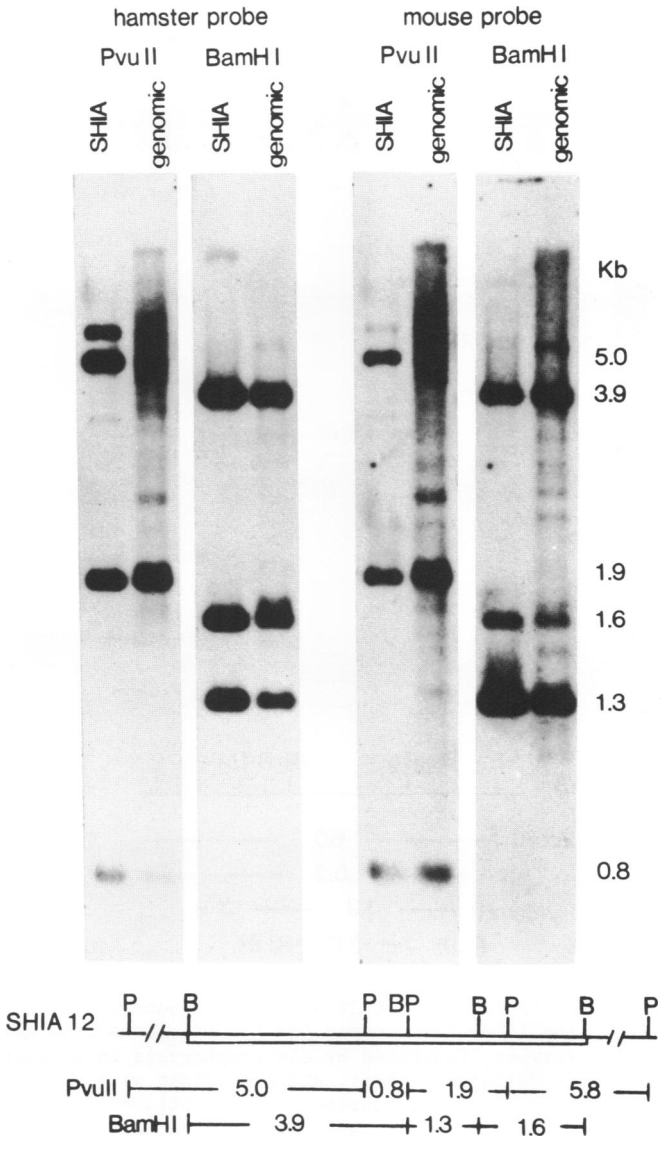

Figure 6. Blot hybridization of Syrian hamster IAP-related sequences. Aliquots of DNAs from λSHIA12 (2 μg, SHIA) and Syrian hamster liver (10 μg, genomic) were digested with PvuII or with BamHI, separated by electrophoresis in an agarose gel, transferred to a nitrocellulose filter, and hybridized with either a Syrian hamster probe (lanes 1-4) or a mouse IAP probe (lanes 5-8) as described in Methods. The probes are described in Figure 5. A partial restriction map of recombinant λSHIA12, containing a hamster IAP sequence element, is shown at the bottom. Fragments generated by digestion of the cloned DNA with PvuII (P) and BamHI (B) are indicated.

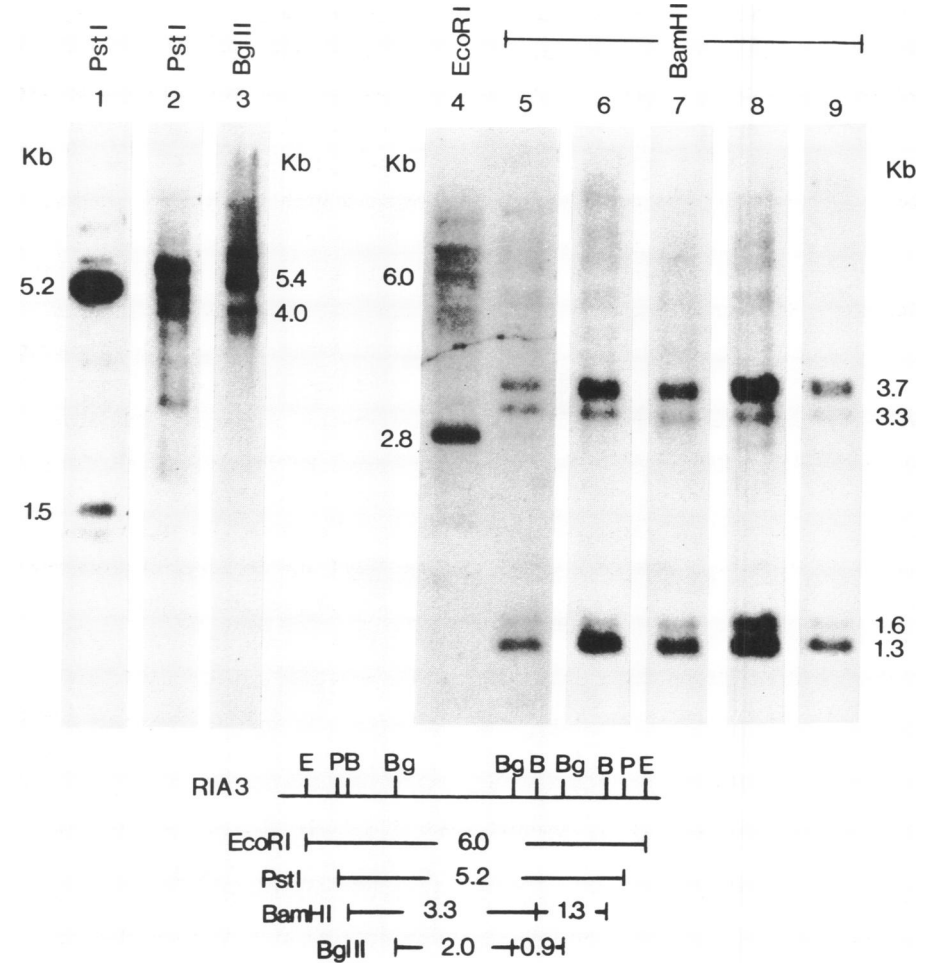

Figure 7. Blot hybridization of rat IAP-related sequences. Aliquots of DNAs from λRIA3 (1 μg, lane 1) or rat genomic DNA (10 μg, lanes 2-9) were digested with the indicated enzymes, separated by electrophoresis in an agarose gel, transferred to a nitrocellulose filter, and hybridized with the 5.2 Kb PstI fragment from pRIA3. Genomic DNA sources were as follows: Lanes 2-5 Sprague-Dawley; lane 6, di/+; lane 7, F344; lane 8, Osborne-Mendel; lane 9, Lewis. A partial restriction map of recombinant λRIA3, containing rat IAP sequences, is shown at the bottom. Fragment generated by digestion of the cloned DNA with EcoRI (E), PstI (P), BamHI (B), and BglII (Bg) are indicated.

clear from this blot that the cloned hamster IAP sequences in λSHIA12 represent one of many similar sequence elements in the genomic DNA. After digestion with PvuII, the two internal fragments from λSHIA12 (1.9 and 0.8 Kb) were also seen as discrete components in genomic DNA (see lane 2). A hetero-

geneous distribution of components was found in the region of the gel corresponding to the two fusion fragments (5.0 and 5.8 Kb in lane 1) derived from λSHIA12. This provides additional evidence that the IAP units in the Syrian hamster genome are interspersed among different flanking sequences. Hybridization of the same blot with a mouse IAP probe (pMIA1) (Fig. 6, lanes 5-8) yielded essentially the same patterns as obtained with the hamster probes.

Hybridization of the rat probe to digests of rat genomic DNA is shown in Figure 7. When genomic DNA was digested with enzymes which cut entirely within the IAP sequence unit in λRIA3 (see Fig. 5), two types of patterns were seen: (a) PstI and BamHI produced discrete bands, some of which corresponded in size to the fragments from isolated clones. For example, a 5.2 Kb PstI fragment such as that in λRIA3 was also detected in genomic DNA blots (compare lanes 1 and 2) although not as the major component. Also in the BamHI pattern (lane 5), two of the four major fragments seen in genomic DNA (3.3 and 1.3 Kb) were those expected on the basis of the λRIA3 restriction map shown in Figure 5. (b) Other enzymes such as BglII and EcoRI which also cut entirely within the IAP unit in λRIA3 produced some prominent bands in genomic blots but these did not correspond in size to the fragments from any of the isolated clones. For instance, λRIA3 contained a 6 Kb EcoRI fragment which did not appear as a prominent component in the genomic DNA digest (lane 4), although a minor 6 Kb component was present. Conversely, none of the cloned rat IAP elements contained an EcoRI fragment corresponding to the prominent 2.8 Kb component in genomic DNA. Similarly, the 2.0 and 0.9 Kb BglII fragments in λRIA3 were not seen as components in the genomic DNA digest (lane 3). Evidently, none of the individual rat IAP sequence elements that we isolated from the library was representitive of a large number of similar units within the rat genome. The observed restriction site polymorphism was consistent with the numerous areas of non-homology seen between individual rat IAP sequences on heteroduplex analysis. Those enzymes which produced the first type of patterns mapped to positions on the rat IAP units which shared homology with the mouse IAP genes and may represent relatively well conserved rat IAP sequences. Those which produced the second type of pattern mapped to regions which had no homology with the mouse genes; these regions may be more hetereogeneous among the rat IAP units.

We compared the BamHI restriction patterns of IAP-related sequences in four inbred rat strains with that obtained from Sprague-Dawley rat (Fig. 7, lanes 5-9). The patterns were very similar with respect to both the major and minor more heterogeneous components.

Earlier we had predicted that Syrian hamster IAP sequence elements would be organized as approximately 7 Kb units (4). Suzuki et al. (5) subsequently reported a size of 7 Kb based on restriction analysis of cloned sequences, and we have now confirmed this estimate by direct heteroduplex measurement. The presence of LTRs, a typical feature of retrovirus gene structure, was demonstrated in Syrian hamster IAP sequences by Suzuki et al. (5). They also reported that the hamster IAP LTRs contained sequences homologous to the mouse IAP LTRs. Clusters of three restriction sites which were identified in the hamster IAP LTRs by Suzuki et al. are found 7 Kb apart in λSHIA12, strongly suggesting that this particular sequence element also contains LTRs. However, a probe containing the entire LTR derived from a cloned mouse IAP gene, λMIA14 (2) did not react with λSHIA12 or with Syrian hamster genomic DNA even under very relaxed hybridization conditions. Both hamster and mouse LTRs do contain a PstI site which may reflect the presence of a limited amount of conserved sequence.

Because of their internal heterogeneity, it was not possible to determine the unit size of the rat IAP sequences or to discern whether they also are flanked by LTRs. Over the >8 Kb mapped in λRIA3, we were not able to identify any repeated clusters of restriction sites. Our mouse IAP LTR probe also failed to react with rat genomic DNA.

Thermal Stability of Hybrids Containing Rat and Syrian Hamster IAP Sequences

We measured the thermal stability of hybrids formed between individual cloned sequences and between cloned sequences and their respective genomic DNA. Figure 8A compares the melting curves for hybrids formed between the rat probe and pRIA3 (self) or rat genomic DNA. Panels B and C show similar experiments carried out with mouse and Syrian hamster probes. Panel D shows melting curves for hybrids formed between the mouse, rat, and Syrian hamster probes and other cloned IAP sequence elements from these species.

The self-hybrids in all cases had a T_m of 84.5°C. Hybrids formed between mouse probe and mouse genomic DNA melted only 1° below that of the self-hybrid (Fig. 8B), indicating that the average sequence divergence within the family of mouse IAP genes is small. Even hybrids with DNA from a deleted/substituted IAP gene, λMIA80, selected as an example of a highly modified mouse IAP element, showed a ΔT_m of only 2° (Fig. 8D).

Hybrids between the rat probe and rat genomic DNA, on the other hand, had a T_m 6° below that of the self-hybrid (Fig. 8A), indicating a large degree of divergence between the rat family members. Similar low T_ms were also characteristic of hybrids formed between the rat probe and several other cloned rat

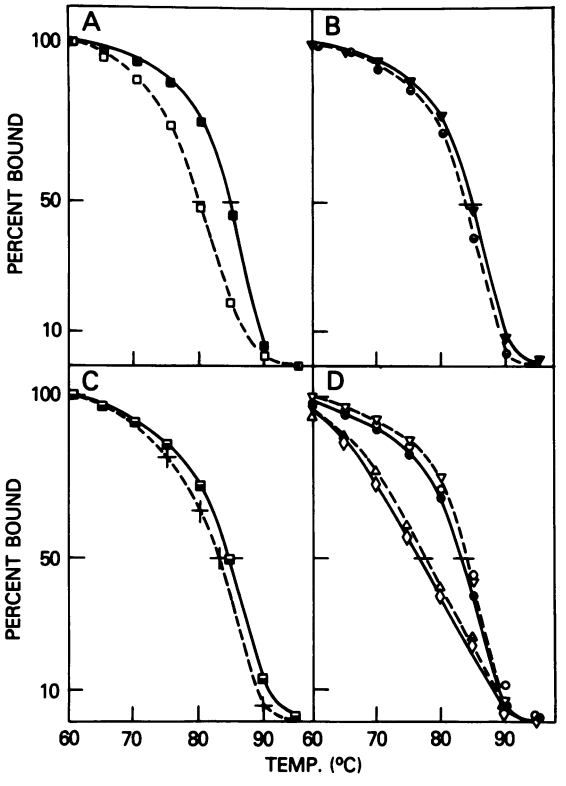

Figure 8. Thermal stability of hybrids containing IAP-related sequences. The probes (shown in Figure 5) were as follows: rat, a 5.2 Kb PstI fragment from pRIA3; mouse, a 5.2 Kb EcoRI/HindIII fragment from pMIA1; and Syrian hamster, a 1:1 mix of the 3.6 and 3.4 Kb PstI fragments from pSHIA12s. A. The rat probe was hybridized with ■, pRIA3 DNA or with □, Sprague-Dawley rat genomic DNA. B. The mouse probe was hybridized with ▼, pMIA1 DNA or with ◒, BALB/c mouse genomic DNA. C. The Syrian hamster probe was hybridized with ▣, pSHIA12-1 plus -2 DNAs or with +, Syrian hamster DNA. D. The mouse probe was hybridized with ▽, λMIA58 or ●, λMIA80 DNA; the Syrian hamster probe was hybridized with ○, λSHIA1 DNA; the rat probe was hybridized with △, λRIA106 or with ◇, λRIA10 DNA.

IAP sequences (Fig. 8D).

Hybrids formed between the hamster probe and hamster genomic DNA again had a T_m only 1.5° below that of the self-hybrid (Fig. 8C). By this criterion, the IAP sequences in the Syrian hamster show a homogeneity similar to that of the IAP sequences in mouse.

Hybridization Patterns of Genomic DNAs from R. Norwegicus and R. Rattus

A comparison of the hybridization patterns of R. norwegicus and R. rattus

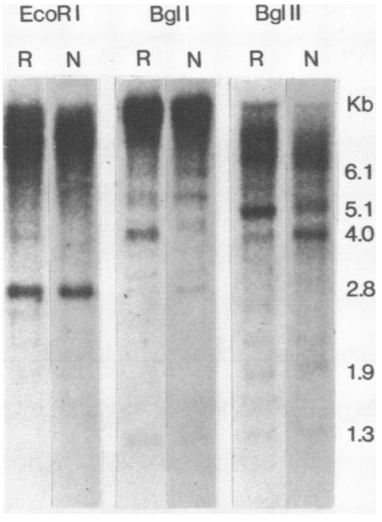

Figure 9. Comparison of the blot hybridization of rat IAP-related sequences in the genomic DNA of Sprague-Dawley strain of R. norwegicus (N) and R. rattus (R). Aliquots of liver DNAs (10 µg) were digested with the indicated enzymes and analyzed as described for Figure 7, except in this case the mouse IAP probe was used.

DNAs is shown in Figure 9. These two species of rat are thought to have diverged 2.3 x 10^6 years ago (10). The two species both have heterogeneous patterns, with a number of common prominent components although differences are also apparent. The heterogeneity of the sequences in R. rattus was confirmed by determining the thermal stability of hybrids formed between the pRIA3 probe and R. rattus genomic DNA (not shown); these melted 8° below the homoduplexes (compared with 6° for R. norwegicus DNA). The similar intensity of reaction of the probe with each DNA on blots along with the small difference in T_ms indicates that the IAP sequence copy number in the two rat species is similar. These data suggest that the IAP sequences were already reiterated in the rat ancestral form before R. norwegicus and R. rattus separated as species and that the sequences have independently diverged in a similar way in each species.

Comparison of IAP Sequences in Syrian Hamster and Chinese Hamster Genomes

In an earlier study (4), hybridization of a mouse IAP probe to Syrian and Chinese hamster DNAs suggested that the organization and copy number of the related sequences in the hamster genera were quite different. We have now used the Syrian hamster probe to examine this question. The hybrids formed between this probe and the Chinese hamster genomic DNA had a T_m 16-20° below

the hybrids formed with homologous Syrian hamster DNA. Thus, the IAP-related sequences present in Chinese hamster are quite divergent from those in the Syrian hamster.

Equal aliquots of genomic DNAs from Syrian and Chinese hamster livers digested with several different restriction enzymes were compared by blot hybridization using pSHIA12 probes. The blots were washed as described in Methods in order to detect divergent sequences with a ΔT_m of -46°. The reaction of Syrian hamster DNA was very strong after a few hours of exposure for autoradiography whereas the reaction with the Chinese hamster DNA even under these very relaxed conditions was barely detectable (not shown). This result suggests that the copy number of IAP-related sequences is much lower in the Chinese hamster genome than in the Syrian hamster genome.

DISCUSSION

Since related but divergent IAP sequences are found in members of at least two families (Muridae and Cricetidae) of the order Rodentia (4,5, this study) this gene family probably arose at least 20 x 10^6 years ago (10). In considering the present results it is important to remember that the properties of any family of sequence elements may be related to the stage in its evolution at which it is analyzed. As has been emphasized by Southern (11) and by Brown and Dover (12), families of closely related sequence elements resulting from amplification and/or homogenization events can subsequently diverge to form internally heterogeneous populations.

In this study we have compared the sequence organization of related multigene families in three evolutionarily distant rodent genomes. The mouse and Syrian hamster IAP units appear to represent relatively homogeneous families of sequence elements as indicated both by restriction enzyme analysis and by the high thermal stability (ΔT_m 1°C) of hybrids between family members. This fact plus the observed divergence between the hamster and mouse IAP sequence elements suggests that fairly recent amplification or homogenization events have occurred independently in the two genomes. In contrast, the rat IAP units represent a more highly diverse family. None of the recombinants we isolated appeared to be a "typical" rat IAP unit as judged by comparison of the restriction patterns of cloned sequences and genomic DNA. Regions of homology between individual rat IAP sequence elements were interrupted by regions of non-homology, and sequences shared between one pair of rat IAP units were not necessarily shared in other combinations. The rat IAP units appear to be made up of a patchwork of different subsets of sequences, including a common

subset related to but divergent from the mouse sequences. The heterogeneity of the individual rat IAP sequence elements and the presence of similar amounts of IAP-related sequence in R. rattus and R. norwegicus are consistent with an amplification of these sequences before divergence of the rat species approximately 2.3 x 10^6 years ago (10). Amplification of the sequences thus seems to have occurred independently and at different times in the evolutionary lineage of at least three genera (Mus [European], Rattus, and Mesocricetus). The genomic DNA patterns for gerbil (Gerbillus) show a moderate number of relatively homogeneous fragments reacting with a mouse IAP probe (4), perhaps indicating an intermediate amplification which may be in progress. There appears to have been no recent amplification of homologous sequences in the genome of Cricetulus (Chinese hamster).

Molecularly cloned members of other dispersed moderately repetitive families have been isolated from the mouse genome and related sequences studied in various rodent genera. Two examples of such families are the MIF-1 and VL30 sequences of mice. Brown and Dover (12) have compared the organization and evolution of the MIF-1 family in Mus and Apodemus, and Heller and Arnheim (13) have demonstrated that sequences homologous to the 1.3 Kb EcoRI fragment of MIF-1 are present in the genomes of Mus caroli, Mus cervicolor, Rattus rattus and Chinese hamster. In these studies the mouse probe was used to characterize the sequence organization in the other genomes. VL30 genes represent a family of endogenous retrovirus-like genes which code for an RNA that can be packaged into murine leukemia viruses (14). Using a mouse VL30 probe, homologous sequences have been identified in the rat genome (15), but shown to be absent in the Chinese hamster (16). The mouse VL30 genes resemble the rat IAP sequences in having regions of homology interspersed with regions of non-homology (14). Individual members of moderately reiterated families of endogenous retroviruses have also been shown to be polymorphic in species other than rodents (17,18).

The related regions between the rat and hamster IAP sequences and the mouse IAP genes as defined by heteroduplex mapping consisted of a 5-6 Kb stretch in which regions of strong and weak homologies were interspersed with non-homologous regions. The homologous regions were ordered in a linear fashion with respect to the mouse genes and regions of non-homology (ranging from 0.5-1.0 Kb) were similar in length in members of a heteroduplex pair, suggesting that the IAP sequences in all three genomes are derived from a common progenitor and that conservation of the linear order and length is important. The regions of "non-homology" may be more apparent than real due

to the limits of the procedures used to detect them, and sequencing may show that a continuum of divergence exists.

Suzuki et al. (5) have found that Syrian hamster IAP sequences are flanked by LTR sequences and reported a homology between mouse IAP LTR sequences and isolated hamster IAP sequences which we have not detected either by heteroduplex analysis or by blot hybridization. The mouse LTR probe used by Suzuki et al. contained 0.9 Kb of flanking sequence in addition to the LTR itself, and this could have contributed to the hybridization they detected. The mouse LTR probe we used also failed to react with rat genomic DNA or cloned rat IAP sequences. Our results suggest that the hamster and rat IAP sequence elements may have species-specific LTRs.

Intracisternal virus-like particles are expressed in embryonic (19-22) and transformed (see references in 4) cells of all three rodent genera from which we have isolated IAP sequences. We have previously shown that the 7 Kb mouse IAP genes are colinear with the 35S genomic IAP RNA (2). Suzuki et al. (5) reported that BHK cells containing intracisternal virus-like "R-particles" (23) yield RNA which reacts with a mouse IAP probe, and we have confirmed this using a cloned Syrian hamster IAP sequence as probe. Similarly, we have found that RNA prepared from rat myeloma cells expressing IAPs (24) reacted with pRIA3 probe, while RNA from IAP-negative rat liver did not. Although these results are suggestive, it remains to be shown whether the sequences we have isolated from the rat and Syrian hamster in fact code for particle-associated RNAs in those species. The mouse IAP genes have been well-characterized and shown to have structural features characteristic of retroviral genes (1,2,25,26). The results of this study and those of Suzuki et al. (5) strongly suggest that the homologous units in the Syrian hamster, and possibly in the rat, also have such properties.

Recent evidence shows that the mouse IAP units can be inserted in new locations in somatic cellular DNA (27-30). This type of insertion in germ line DNA, perhaps favored by the expression of intracisternal particles in embryos such as seen in the mouse (19-22) and Syrian hamster (31), could result in gene amplification over an evolutionary time scale. The amplification process could have occurred by chance in the various genomes at different times.

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