Genetics of Intracisternal-A-Particle-Related Envelope-Encoding Proviral Elements in Mice

FRANK U. REUSS,¹ WAYNE N. FRANKEL,² KAZUO MORIWAKI,³
TOSHIHIKO SHIROISHI,³ AND JOHN M. COFFIN^{1*}

*Department of Molecular Biology and Microbiology, Tufts University School of Medicine,
Boston, Massachusetts 02111¹; Jackson Laboratory, Bar Harbor, Maine 04609²;
and National Institute of Genetics, Mishima, Shizuoka, Japan³*

Received 11 March 1996/Accepted 2 June 1996

Intracisternal-A-particle-related envelope-encoding (IAPE) proviral elements in the mouse genome encode and express an envelope-like protein that may allow transmission of IAPEs as infectious agents. To test IAPE mobility and potential transmission in mice, we have analyzed the distribution of IAPE elements in the genomes of *Mus spretus* and *Mus musculus* inbred strains and wild-caught animals. Potential full-length (IAPE-A) proviral elements are present as repetitive copies in DNA from male but not female animals of *M. musculus* inbred strains and *Mus musculus castaneus*. Analysis of IAPE-cellular junction fragments indicates that fixation of most IAPEs in the germ line occurred in *M. musculus* and *M. spretus* after speciation but before *M. musculus* inbred strains were derived.

Intracisternal A-particles (IAPs) are retrovirus-like, noninfectious particles found in the endoplasmic reticula of a variety of mouse cell types (9). IAPs are encoded by a large number of provirus-like elements in the mouse genome. These IAP elements contain *gag* and *pol* genes but lack the *env* gene. The absence of IAP envelope glycoproteins is the most obvious cause for the loss of IAP infectivity. Other incapacitating problems include the defective maturation of the *gag* polyprotein (12) and the hydrophobic N terminus of the Gag protein, which is implicated in the assembly of particles at the membranes of the endoplasmic reticulum (13).

The intracisternal-A-particle-related envelope-encoding (IAPE) group of retroviral elements in *Mus musculus* contains a reading frame with close resemblance to retroviral *env* genes (17). IAPE transcripts include a 2.8-kb spliced subgenomic *env* mRNA. Antisera directed against the IAPE *env* gene product specifically detect N-glycosylated proteins of 91 kDa or less in cell lines positive for IAPE RNAs (16). IAPE envelope proteins thus may allow the assembly of an as-yet-unidentified infectious IAPE virus.

IAPE proviral elements exist in approximately 200 copies per *M. musculus* genome and can be divided into at least three groups based on the size of the internal proviral region after *Eco*RI restriction digestion (Fig. 1) (17). The 7.5-kb IAPE subgroup (IAPE-A) potentially represents intact IAPE proviruses whereas the smaller (4.0-kb [IAPE-B] and 3.7-kb [IAPE-C]) elements have been shown to lack large parts of the *gag* and *pol* genes. Each of these subgroups consists of approximately 30 to 50 proviral elements (17). A number of IAPE elements differ in size from the types described above and may represent either proviruses with less common truncations or restriction site polymorphisms. Despite the presence of apparently full-length proviruses, Northern blot RNA analysis of two mouse cell lines failed to detect IAPE RNAs in the size range corresponding to intact IAPEs (IAPE-A) (16), while smaller transcripts were detectable. Thus, the transcription of intact full-length IAPE proviral elements might be tightly regulated,

perhaps restricted to certain cell types or stages during mouse development.

The presence of infectious endogenous retrovirus in a mouse population results in a diversity in both the number and the location of proviruses within the host genome (3, 8). To examine the extent of genetic diversity of IAPEs, we analyzed IAPE subsets in several mouse laboratory inbred strains as well as two species of wild mice. The pattern of IAPE loci was identified by the cellular flanking regions. In such an analysis, loci present in all samples would indicate integration at an early time during the evolution of the genus *Mus* whereas loci restricted to certain strains are likely to be late acquisitions.

We extended our previous analysis of mouse IAPE subtypes to a number of male and female animals of several *M. musculus* laboratory inbred strains and to the Asian house mouse *Mus musculus castaneus*, obtained from different locations. Most of today's mouse inbred strains are derived from the west European house mouse *Mus musculus domesticus* and the Asian *Mus musculus molossinus* or *M. musculus castaneus*. *Mus spretus* from Spain was tested as example of a separate species. DNA (10 µg) isolated from liver tissue (11) was digested with *Eco*RI and separated on a 0.8% agarose gel. The DNA within the dried gel, or "unblot," was hybridized to ³²P-labeled oligonucleotide FR2871/20 (5'-GCATCAGCCAGATCTGCTGA-3') as described previously (18). FR2871/20 represents the antisense strand of a conserved region in the IAPE *env* gene (nucleotides nt 2871 to 2890 of IAPE-1) that is found in all IAPE cDNAs (IAPE-1, IAPE-4, and IAPE-28), where this part has been sequenced (17) (Fig. 1).

We were able to distinguish in *M. musculus* the three distinct IAPE types that we described previously (17). However, our results show that in all four cases in which DNA samples from males and females of the same *M. musculus* inbred strain were used, the repetitive 7.5-kb (IAPE-A) type was found only for the male (Fig. 2A). This genetic sexual dimorphism is also found in males and females of other *M. musculus* inbred strains, for which matched samples for the opposite sexes were not included (Fig. 2) and a selection of *M. musculus castaneus* wild mice from Asia (Fig. 2B). Single IAPE-A elements may be present in females of certain strains (C57BL/6J [Fig. 2B]). The smaller (4.0-kb [IAPE-B] and 3.7-kb [IAPE-C]) IAPEs with

* Corresponding author. Phone: (617) 636-6528. Fax: (617) 636-8086. Electronic mail address: jcoffin_par@opal.tufts.edu.

FIG. 1. Schematic representation of IAPE elements in the mouse genome. IAPE elements are shown as straight lines and cellular DNA is shown as wavy lines. Internal IAPE fragments after *Eco*RI digestion fall into three size ranges and are designated IAPE-A, IAPE-B, and IAPE-C. Digestion with *Bam*HI or *Pvu*II creates junction fragments between IAPE elements and flanking cellular sequences. The IAPE internal fragments and the 3' junction fragments were detected by hybridization with an IAPE *env*-specific oligonucleotide. LTR, long terminal repeat.

known deletions in the *gag* and *pol* genes are present in equal numbers in both sexes of *M. musculus* inbred strains and in *M. musculus castaneus* samples. *M. spretus* is closely related to *M. musculus* and commonly used for interspecies crosses (10). IAPE proviral elements were also present in *M. spretus* (Fig. 2B). However, our DNA sample from male *M. spretus* differed from samples from *M. musculus* males by the complete lack of 3.7-kb (IAPE-C) IAPE elements. The 7.5- and 4.0-kb IAPes (IAPE-A and IAPE-B, respectively) are predominant.

The presence of infectious endogenous retroviruses in a mouse population is correlated with a high genetic variability in the number and location of proviral loci among inbred strains, subspecies, and species. Such variability has been observed with murine leukemia virus (3) and mouse mammary tumor virus (8). It is commonly visualized as restriction length polymorphism of cellular sequences flanking individual proviral loci. To examine potential differences in the locations of IAPE proviral elements, we analyzed the size distribution of 3'-junction fragments between IAPes and cellular DNA after *Bam*HI or *Pvu*II digestion. These enzymes cut at positions 2698 (*Bam*HI) and 2812 (*Pvu*II) of cDNA clone IAPE-1 (17) and are expected to create 3'-junction fragments of 1.4 kb (*Bam*HI) and 1.3 kb (*Pvu*II) of viral DNA joined to flanking cell DNA (Fig. 1). Digested mouse genomic DNAs were processed and hybridized as described above.

All *M. musculus* samples exhibited the genetic sexual dimorphism previously seen with IAPE-A internal fragments. A repetitive 3'-junction fragment of 2.1 kb was seen in *Bam*HI-digested DNA from *M. musculus* inbred-strain males that was not present in females of the same strain (Fig. 3). After *Pvu*II digestion, a major repetitive junction fragment of 3.2 kb and a minor repetitive fragment of 3.7 kb were observed in male animals of *M. musculus* inbred strains, *M. musculus molossinus* from Japan, and *M. musculus castaneus* from Taiwan, Malaysia, or Indonesia (Fig. 4). The presence of repetitive junction fragments indicates that one single IAPE element, after initial integration into host DNA, has been subsequently amplified together with flanking cellular sequences. The singleness of the

repetitive junction region resulting from *Bam*HI digestion makes it likely that the minor *Pvu*II set reflects a sequence polymorphism in some IAPE-A copies or flanking regions rather than a second independent integration-amplification event. As both IAPE-A elements and the repetitive flanking regions are male specific, we conclude that IAPE-A elements have been amplified as part of a larger cellular region of the *M. musculus* Y chromosome.

These repetitive fragment patterns were not observed in DNA sample from male *M. spretus* after analysis of cellular sequences flanking IAPes with either *Bam*HI or *Pvu*II (Fig. 3 and 4). All IAPE-A and IAPE-B loci in *M. spretus* are likely to represent independent integration events and have not been subjected to a subsequent amplification.

The large number of IAPE elements in the mouse genome makes the resolution of single copy junction regions problematic, in particular in the larger size range, such that some differences between strains may not be detected. In *Bam*HI-digested samples, comparison among *M. musculus* inbred strains showed a large number of IAPE loci common among all strains (Fig. 3). However, some IAPE junction fragments were restricted to certain inbred strains and were not found in others. The presence of strain-specific IAPE loci may reflect either a recent IAPE transposition during inbreeding, a different genetic background of founder animals, or restriction site polymorphisms. A larger number of differences was found between North American *M. musculus* inbred strains and an *M. musculus* inbred strain from Peru. Most junction fragments differ for *M. spretus* and *M. musculus* strains. These results suggest that replicating IAPE elements were present in the common ancestor of *M. musculus* and *M. spretus*. Fixation of most IAPes in the germ line occurred in *M. musculus* and *M. spretus* after speciation but before *M. musculus* inbred strains were generated.

It has previously been shown that the Y chromosome of *M. musculus* inbred strains is derived from the Asian *M. musculus molossinus* in certain strains but inherited from the west European *M. musculus domesticus* in others (15, 19). In our

FIG. 2. Internal IAPE fragments in *Eco*RI-digested mouse DNAs. (A) Results for *M. musculus* inbred strains. (B) Results for *M. musculus* inbred strains (lanes C57BL/6J to AKR/J), *M. musculus castaneus* (lanes 1 to 5), and *M. spretus* (*M. spret.*). Genomic DNA from mouse specimens (10 μ g each) was *Eco*RI digested, separated on a 0.8% agarose gel, and dried. The dried gel was hybridized to IAPE *env*-specific oligonucleotide FR2871/20 ($>0.5 \times 10^6$ cpm/ml) at 50°C in a solution of 5 $\times$ SSPE (0.75 M NaCl, 50 mM NaH₂PO₄, 20 mM EDTA, pH 7.4)–0.1% sodium dodecyl sulfate (SDS)–10 μ g of salmon sperm DNA per ml and washed at room temperature in 2 $\times$ SSC (0.3 M NaCl, 30 mM sodium citrate, pH 7.0)–0.1% SDS. The sex (M, male; F, female) for each sample is identified below the lanes. The mouse strains from which DNA samples were obtained are indicated above the lanes. Lanes 1 to 5, *M. musculus castaneus* wild mice from Malaysia (specimen MG-5058), Bandung (Indonesia) (MG-0453), Hemei (Taiwan) (MG-5062), Taichung (Taiwan) (MG-0166), and Taidong (Taiwan) (MG-0265), respectively. The MG numbers are the serial numbers of wild-mouse specimens at the National Institute of Genetics, Mishima, Japan. All *M. musculus* inbred strains were purchased from The Jackson Laboratory (Bar Harbor, Maine).

FIG. 3. IAPE-cellular junction fragments in *Bam*HI-digested mouse DNAs. Genomic DNA from indicated mouse specimens (10 μ g each) was *Bam*HI digested, separated on a 0.8% agarose gel, and hybridized to IAPE *env*-specific oligonucleotide FR2871/20. The sex (M, male; F, female) for each sample is identified below the lanes. *M. spret.*, *M. spretus* strain from Spain; Peru(Co), *M. musculus* inbred strain PERC/Ei (Peru-Coppock) from Peru. All other strains are *M. musculus* inbred strains.

study we have analyzed male animals representing the Asian *M. musculus molossinus* Y chromosome types 1A (C57BL/6J, C58/J, SM/J, BALB/cJ, and CE/J) and 2 (C3H/HeJ and DBA/2J) as well as the *M. musculus domesticus* types 3 (SWR/J) and 5 (AKR/J). Repetitive IAPE-A elements were present in male animals of all these strains. This distribution indicates that the IAPE-A elements are also present on the Y chromosome of *M. musculus domesticus*. We therefore conclude that the initial IAPE-A proviral element on the Y chromosome had been established in the germ line and amplified before the separation of the *M. musculus molossinus*, *M. musculus castaneus*, and *M. musculus domesticus* but after speciation from *M. spretus*.

The combined data from our IAPE subtype and IAPE locus analyses suggest the following sequence of events during mouse-IAPE coevolution (Fig. 5). Replicating IAPE-A and IAPE-B were present in the ancestral *Mus* population before the evolutionary separation of *M. musculus* and *M. spretus* $\approx$ 2 million years ago (14). However, the differences in the position of IAPE loci within the genome between these two species indicate that most IAPE loci were established in the host germ line only after separation of the species. While IAPes in *M. spretus* did not undergo major detectable changes, two developments are apparent in *M. musculus*: (i) integration of a copy of IAPE-A on the Y chromosome and its subsequent amplification as part of the host genome before separation of

FIG. 4. IAPE-cellular junction fragments in *PvuII*-digested mouse DNAs. Genomic DNA from indicated mouse specimens (10 μ g each) was *PvuII* digested, separated on a 0.8% agarose gel, and hybridized to IAPE *env*-specific oligonucleotide FR2871/20. The sex (M, male; F, female) for each sample is identified below the lanes. Lanes 1 to 9, *M. musculus molossinus* wild mice from Nirayoma (Shizuoka, Japan) (specimen MG-0220), Mishima (Shizuoka, Japan) (MG-5094), Hakozaiki (Fukuoka, Japan) (MG-0459), and Ashiro (Iwate, Japan) (MG-0230) and *M. musculus castaneus* wild mice from Malaysia (specimen MG-5058), Bandung (Indonesia) (MG-0453), Hemei (Taiwan) (MG-5062), Taichung (Taiwan) (MG-0166), and Taidong (Taiwan) (MG-0265), respectively. AKR/J to SM/J are *M. musculus* inbred strains. *M. spret.*, *M. spretus* (Spain).

M. musculus castaneus, *M. musculus molossinus*, and *M. musculus domesticus* (≈ 0.8 million years ago) and (ii) generation of IAPE-C elements through a truncation(s) of the *gag* and *pol* genes and their integration into a number of distinct genomic

FIG. 5. Inferred sequence of events during coevolution of IAPE elements and mice. The IAPE subsets (IAPE-A, IAPE-B, and IAPE-C) detected in our experiments are indicated in parentheses after each species or subspecies. The presence of amplified IAPE-A proviral elements on the Y chromosome (A^Y) is indicated. The events during IAPE evolution, inferred from the data, are indicated by numbers. Broken lines represent *M. musculus* subspecies not tested in this study. The diagram is adapted from reference 14. 1, presence of replicating IAPE-A and IAPE-B elements; 2, generation of IAPE-C elements; 3, fixation of most or all IAPE loci; 4, amplification of IAPE-A Y-chromosomal element.

loci. The IAPE-C elements thus represent the most recent of the three major IAPE types.

Strong suppression of crossing over between major parts of the X and Y chromosomes permitted accumulation of several types of repetitive sequences in the mouse Y chromosome (4, 6). One of these, MuRVY, resembles murine leukemia viruses in sequence and structure and is clearly distinct from IAPes (7). A recent report, published during preparation of our manuscript, describes the close physical association and coamplification of MuRVY and IAPE-Y (IAPE-A in our nomenclature) proviral elements on the *M. musculus* Y chromosome (2). These results and the strict linkage of repetitive IAPE-A elements to the male sex imply that the repetitive IAPE-A elements are located in the differential (nonpseudoautosomal) region on the long arm of the *M. musculus* Y chromosome (1).

IAPE-A elements are likely to represent full-length IAPE elements and could potentially encode a yet-unknown, infectious IAPE virus. The expression of envelope-like IAPE proteins described in our earlier work (16) was detected in the absence of IAPE-A RNA and thus is likely to involve IAPE-B or IAPE-C elements. These elements are, however, unable to provide the IAPE *gag* and *pol* gene products. With IAPE-A as the only possible source of these proteins, IAPE virus production may occur predominantly in *M. musculus* males. Alternatively, coexpression of multiple defective IAP or IAPE genomes in a cell could lead to the production of infectious virus particles in the absence of complete genomes.

Although a large number of apparently full-length IAPE-A proviruses are present on *M. musculus* Y chromosomes, an earlier analysis of the neuroblastoma cell line NH15-CA2 (16, 17) demonstrated that the presence of a Y chromosome is not sufficient for IAPE-A transcription, even though mRNAs derived from autosomal or X chromosomal IAPE-B and IAPE-C elements are abundant. The physical condensation status of the Y chromosome is likely to be an additional critical factor in IAPE-A transcription. The mammalian Y and X chromosomes are condensed and transcriptionally inactive during male meiosis (see reference 6 for a review). Guttenbach and colleagues (5) have determined the condensation status of the mouse Y chromosome in other cell types and detected Y chromosome decondensation only in Sertoli cells of the testes but not interphase nuclei of several somatic tissues. Thus, it is tempting to speculate that Y chromosome condensation may restrict *M. musculus* IAPE-A expression to the mouse testis.

We are grateful to U. Klingmüller for help with the preparation of figures.

J.M.C. is American Cancer Society Research Professor of Molecular Biology and Microbiology. This work was also supported by National Cancer Institute award R35CA44385 to J.M.C. and a Leukemia Society of American Fellowship to F.U.R.

REFERENCES

1. Eicher, E. M., S. J. Phillips, and L. L. Washburn. 1983. The use of molecular probes and chromosomal arrangements to partition the mouse Y chromosome into functional regions, p. 57-71. In A. Messer and I. H. Porter (ed.), Recombinant DNA and medical genetics. Academic Press, New York.
2. Fennelly, J., K. Harper, S. Laval, E. Wright, and M. Plumb. 1996. Coamplification of tail-to-tail copies of MuRVY and IAPE retroviral genomes on the *Mus musculus* Y chromosome. Mamm. Genome 7:31-36.
3. Frankel, W. N., J. P. Stoye, B. A. Taylor, and J. M. Coffin. 1990. A linkage map of endogenous murine leukemia proviruses. Genetics 124:221-236.
4. Graves, J. A. M. 1995. The origin and function of the mammalian Y chromosome and Y-borne genes—an evolving understanding. Bioessays 17:311-321.
5. Guttenbach, M., M. Schmid, A. Jauch, and P. Vogt. 1989. The Y chromosome of the mouse is decondensed in Sertoli cells. Chromosoma 97:429-433.
6. Handel, M. A., and P. A. Hunt. 1992. Sex-chromosome pairing and activity during mammalian meiosis. Bioessays 14:817-822.
7. Hutchison, K. W., and E. M. Eicher. 1989. An amplified endogenous retro-

- viral sequence on the murine Y chromosome related to murine leukemia viruses and viruslike 30S sequences. *J. Virol.* **63**:4043–4046.
8. Kozak, C., G. Peters, R. Pauley, V. Morris, R. Michalides, J. Dudley, M. Green, M. Davisson, O. Prakash, A. Vaidya, J. Hilgers, A. Verstraeten, N. Hynes, H. Diggelmann, D. Peterson, J. C. Cohen, C. Dickson, N. Sarkar, R. Nüsse, H. Varmus, and R. Callahan. 1987. A standardized nomenclature for endogenous mouse mammary tumor viruses. *J. Virol.* **61**:1651–1654.
 9. Kuff, E. L., and K. K. Lueders. 1988. The intracisternal A-particle gene family: structure and functional aspects. *Adv. Cancer Res.* **51**:183–276.
 10. Lueders, K. K., and W. N. Frankel. 1994. Mapping of mouse intracisternal A-particle proviral markers in an interspecific backcross. *Mamm. Genome* **5**:473–478.
 11. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
 12. Marciani, D. J., and E. L. Kuff. 1973. Isolation and partial characterization of the internal structural proteins from murine intracisternal A-particles. *Biochemistry* **12**:5075–5083.
 13. 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.
 14. Morita, T., H. Kubota, K. Murata, M. Nozaki, C. Delarbre, K. Willison, Y. Satta, M. Sakaizumi, N. Takahata, G. Gachelin, and A. Matsushiro. 1992. Evolution of the mouse t haplotype: recent and worldwide introgression to *Mus musculus*. *Proc. Natl. Acad. Sci. USA* **89**:6851–6855.
 15. Nagamine, C. M., Y. Nishioka, K. Moriwaki, P. Boursot, F. Bonhomme, and Y. F. Lau. 1992. The musculus-type Y chromosome of the laboratory mouse is of Asian origin. *Mamm. Genome* **3**:84–91.
 16. Reuss, F. U. 1992. Expression of intracisternal A-particle-related retroviral element-encoded envelope proteins detected in cell lines. *J. Virol.* **66**:1915–1923.
 17. Reuss, F. U., and H. C. Schaller. 1991. cDNA sequence and genomic characterization of intracisternal A-particle-related retroviral elements containing an envelope gene. *J. Virol.* **65**:5702–5709.
 18. Stoye, J. P., W. N. Frankel, and J. M. Coffin. 1991. DNA hybridization in dried gels with fragmented probes: an improvement over blotting techniques. *Technique* **3**:123–128.
 19. Tucker, P. K., B. K. Lee, B. L. Lundrigan, and E. M. Eicher. 1992. Geographic origin of the Y chromosomes in “old” inbred strains of mice. *Mamm. Genome* **3**:254–261.