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Mouse *U2af1-rs1* Is a Neomorphic Imprinted Gene

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The mouse *U2af1-rs1* gene is an endogenous imprinted gene on the proximal region of chromosome 11. This gene is transcribed exclusively from the unmethylated paternal allele, while the methylated maternal allele is silent. An analysis of genome structure of this gene revealed that the whole gene is located in an intron of the *Murr1* gene. Although none of the three human *U2af1*-related genes have been mapped to chromosome 2, the human homolog of *Murr1* is assigned to chromosome 2. The mouse *Murr1* gene is transcribed biallelically, and therefore it is not imprinted in neonatal mice. Allele-specific methylation is limited to a region around *U2af1-rs1* in an intron of *Murr1*. These results suggest that in chromosomal homology and genomic imprinting, the *U2af1-rs1* gene is distinct from the genome region surrounding it. We have proposed the neomorphic origin of the *U2af1-rs1* gene by retrotransposition and the particular mechanism of genomic imprinting of ectopic genes.

Genomic imprinting is defined as the monoallelic and parent-specific mode of expression of a small number of genes. Embryonic development in mammals depends on imprinted genes, and abnormal development occurs in diploid zygotes that carry only a maternal or paternal set of genomes (40). Each parental allele must be discriminated in all cells to cause these phenomena. Little is known about the discriminating mark or molecular mechanism of imprinting, but DNA methylation is thought to play an important role (4, 5, 9). Allele-specific methylation has been observed in all the endogenous imprinted genes tested so far (33), and some inherit their methylation status from one gamete (1, 22, 42, 46, 49).

Some common features have been observed in the genome structures of endogenous imprinted genes reported to date. For example, imprinted genes often contain direct repetitive sequences (31). A tendency to have few and small introns is common (14, 19). Most of the imprinted genes seem to cluster into certain chromosomal domains, for 7 (or possibly 10) of the imprinted mouse genes map on two distinct domains of chromosome 7 (2). In human, these two domains are located on chromosomes 11 and 15. One of them contains five genes (*H19*, *Igf2*, *Ins2*, *Mash2*, and *p57^{KIP2}*), and the other contains at least two genes (*Snrpn* and *Znf127*) in mouse and five genes (*SNRPN*, *ZNF127*, *PAR1*, *PAR5*, and *IPW*) in human (2, 44, 48). Colocalization of the imprinted genes implies that some mechanisms related to genomic imprinting may function over a long chromosomal region, for example, over several hundred kilobases.

One of the mouse endogenous imprinted genes, *U2af1-rs1* (*SP2*), was isolated by the restriction landmark genomic scanning (RLGS) method, which can display allele-specific methylation of the genome (15, 18). This gene on chromosome 11 is transcribed exclusively from the unmethylated paternal allele and the methylated maternal allele is silent in all the adult tissues that have been analyzed so far (16, 18). This gene is a peculiar example of imprinted genes because it has related sequences elsewhere in the genome. Three examples in mice, the *U2af1-rs1*, *Mash2*, and *Ins* genes, have been reported. These related genes are not clustered in the genome. For instance, *U2af1-rs1* is located on chromosome 11, whereas its related gene *U2af1-rs2* is assigned to chromosome X (47). Interestingly, while both of the *Ins* genes are imprinted (10), *U2af1-rs2* is transcribed from both alleles and thus is not imprinted (47). Although the *Mash2* gene is imprinted (11), *Mash1* is not because *Mash1* heterozygous (+/−) mice do not show any phenotypic characteristics but *Mash1* knockout (−/−) mice do (12). Analyses of these genes will provide clues for elucidating not only the *cis*-acting region essential for imprinting, but also the origin of imprinted genes.

In this study, we examined the genome structure around the mouse endogenous imprinted gene *U2af1-rs1*. We found that it is located within an intron of the *Murr1* gene, which is not imprinted. Taken together with the results from analyses of homology to the human chromosome and methylation status around the *U2af1-rs1* gene, we propose the neomorphic origin of the mouse imprinted gene *U2af1-rs1* by retrotransposition.

MATERIALS AND METHODS

Plaque hybridization and isolation of cDNAs. The neonatal mouse cDNA library used in this study was a lambda gt10 library. Yeast artificial chromosome (YAC) clone 11/SP2#8 (30) and P1 clones were obtained from commercial sources, Research Genetics Inc. (Huntsville, Ala.) and Genome Systems Inc. (St. Louis, Mo.), respectively. Radiolabelled DNA probes were generated from P1 or pulsed-field-isolated YAC clone 11/SP2#8 and synthesized by the random hexamer method (8). Hybridization was performed in a buffer containing 6× SSC (6× SSC is 0.9 M NaCl and 0.09 M sodium citrate), 0.1% sodium dodecyl sulfate, 10× Denhardt's solution (2% each bovine serum albumin, Ficoll, and polyvinylpyrrolidone), 0.5% sodium pyrophosphate, and 100 μg of salmon testis DNA per ml. The probe was preannealed with an excess amount of mouse liver DNA to quench repeat hybridization (37). Subsequent screening of the clones of interest was followed by purification of the DNA fragments and subcloning into plasmid vectors for sequencing. The isolated clones were confirmed by Southern analyses (41) of mouse genomic DNA and *Saccharomyces cerevisiae* DNA harboring 11/SP2#8.

Pulsed-field mapping. Agarose plugs containing mouse or yeast cells were prepared by standard methods (43). Chromosomal DNAs were digested with restriction enzymes and resolved by pulsed-field gel electrophoresis (PFGE) using the contour-clamped homogeneous electric field system (Bio-Rad Laboratories). Filters prepared by transferring DNA from gels were hybridized with cDNA fragments of the *U2af1-rs1*, *Murr1*, or *Murr2* gene to determine their relative map positions. The probes were as follows: *U2af1-rs1*, *Not*I-*Eco*RV or *Sma*I-*Not*I fragment (18); *Murr1*, *Bam*HI-*Eco*T22I, *Bam*HI-*Pst*I, or *Pst*I-*Eco*T22I

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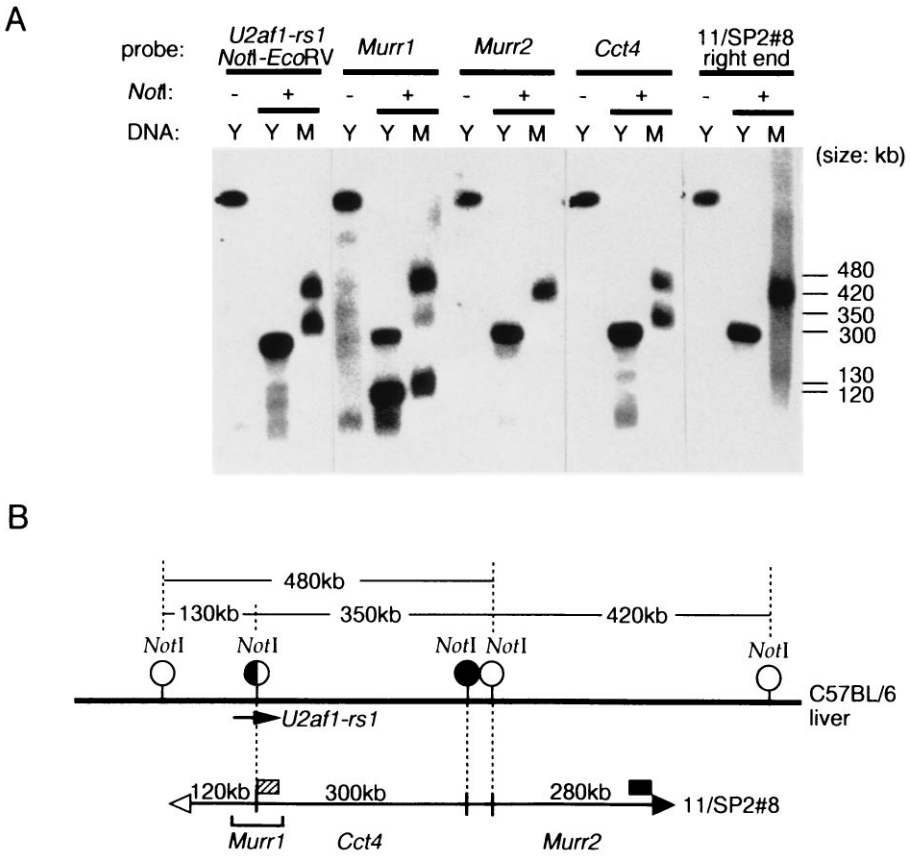

FIG. 1. Physical mapping of the *Murr* genes on YAC clone 11/SP2#8. (A) Southern hybridization analysis using either *U2af1-rs1*, *Cct4*, *Murr1*, or *Murr2* cDNA as a probe. DNA samples were from yeast carrying YAC 11/SP2#8 (Y) and female B6 liver cells (M). (B) Location of the genes in 11/SP2#8. The location and direction of *U2af1-rs1* transcription (arrow), *U2af1-rs1 Not*I-*Eco*RV and YAC right-end fragments used for probes (striped and solid boxes, respectively), and methylated, unmethylated, and half-methylated *Not*I sites in B6 liver cells (solid, open, and half-solid lollipops, respectively) are indicated.

fragment of its cDNA (see Fig. 2A); *Murr2*, an approximately 600-bp fragment amplified by reverse transcriptase PCR (RT-PCR) with the same pair of primers used in the expression analysis (see Fig. 5); and *Cct4*, a 191-bp fragment amplified by RT-PCR as described elsewhere (30). The DNA fragments corresponding to the right and left ends of the YAC insert were isolated by inverse PCR as described previously (20).

Analysis of structure of the *Murr1* intron. We amplified the *Murr1* intron containing *U2af1-rs1* by long and accurate PCR (LA-PCR) (3, 6) using two pairs of primers. LA-PCR was performed according to the instructions of the manufacturer (Takara, Kyoto, Japan). The pair of primers used to amplify the upstream region of *U2af1-rs1* was 5′-AAGACTGCTCAGACAAGTG-3′ and 5′-GGCTCTTCCAAAACTTGGAGATGAC-3′. The other pair, used to amplify the downstream region, was 5′-CAGGAAACAGCTATGACCTTCCATACAGTTTAAGCTGG-3′ and 5′-CAGGAAACAGCTATGACCATGGGTGGCAAGTCCCTG-3′. The amplified fragments from the P1 clones were cloned into plasmid vectors and/or subjected to restriction mapping and sequencing analyses. The sequences thus determined were compared to the cDNA sequence of *Murr1*, and the junctions between exons and the intron were determined.

Chromosomal assignment of the human *MURR1* gene. A cDNA fragment of human *MURR1* was isolated from adult leukocytes by RT-PCR, and primers for the reaction were 5′-GACACTTTCCACGGGTACC-3′ and 5′-ACAGCGTCTTCAGAATTTGG-3′. The pair of primers used to amplify the genomic human *MURR1* locus was 5′-GACACTTTCCACGGGTACC-3′ and 5′-CCCCCTCATCTTTGCCAGA-3′. The hybrid cell panel used in this study is a series of 23 human × mouse A9 or Chinese hamster hybrid cell clones each containing a single human chromosome (26). Chromosomal DNAs prepared from the hybrid cell clones were analyzed by PCR, and 40 cycles of the reaction (95°C for 1 min, 60°C for 1 min, and 72°C for 1 min) were employed.

RT-PCR analysis of gene expression. Four kinds of mouse strains were used in this analysis: C57BL/6 (B6) and PWK and the F_1 mice obtained by reciprocal crosses of them, (PWK × B6)F_1 (referred to as PBF1) and (B6 × PWK)F_1 (BPF1). cDNAs from neonatal mice were prepared and RT-PCR was performed by methods reported previously (17). The primers used for *Murr1* and *Murr2* were as follows: *Murr1*, 5′-CAGGAAACAGCTATGACCATGGGTGGCAAGTCCCTG-3′ and 5′-TGTAAAACGACGGCCAGTGGTAACACCAGTGGGCAAAC-3′; *Murr2*, 5′-TGTAAAACGACGGCCAGTAGAAGATATTGGTGGTCG-3′ and 5′-CAGGAAACAGCTATGACCTACTGGGGACTGAATGGG-3′. Polymorphisms between B6 and PWK were found by sequencing of the RT-PCR products, and the 5′ parts of the primers were used for specific sequencing primers. The *Murr1* RT-PCR products were subjected to *Bsp*HI digestion, and the *Murr2* products were purified by agarose gel electrophoresis and then sequenced. PCR was performed for 30 cycles, and under these conditions the amplification was found to be quantitative and sensitive to both alleles (data not shown).

Methylation analysis of DNA. Long-range analysis of the methylation status was performed by using PFGE, similar to the pulsed-field mapping method described above. Methylation analysis by Southern blotting following conventional agarose gel electrophoresis was performed as described previously (18). The probes were prehybridized with an excess amount of mouse liver DNA to quench repeat hybridization (37). Allelic analyses of methylation were performed by a PCR-based strategy (see Fig. 7A). Chromosomal DNAs isolated from PBF1 or BPF1 liver cells were subjected to digestion by *Hpa*II followed by PCR with a pair of primers (5′-TAAAGCCCATAAGCTATGGG-3′ and 5′-CAGTCTTACAGAAGTCTCTG-3′) for site 2/*Hpa*II. For the *Sma*I/*Hpa*II site, oligonucleotides 5′-TGCTGGCAGCTCACCTTC-3′ and 5′-CAGAACAAATGAAAGGAATG-3′ were used. The allelism of methylation of the *Hpa*II site was determined by sequencing the PCR products and checking the polymorphic nucleotides.

Nucleotide sequence accession numbers. The *Murr1* and *Murr2* cDNA sequences will appear in the DDBJ database under accession no. D85430 and D85434, respectively. The sequences around the exon-intron junctions of *Murr1* have been given DDBJ accession no. D85431 (the 5′ side) and D85432 (the 3′ side), respectively. A cDNA fragment of human *MURR1* was sequenced and given DDBJ accession no. D85433.

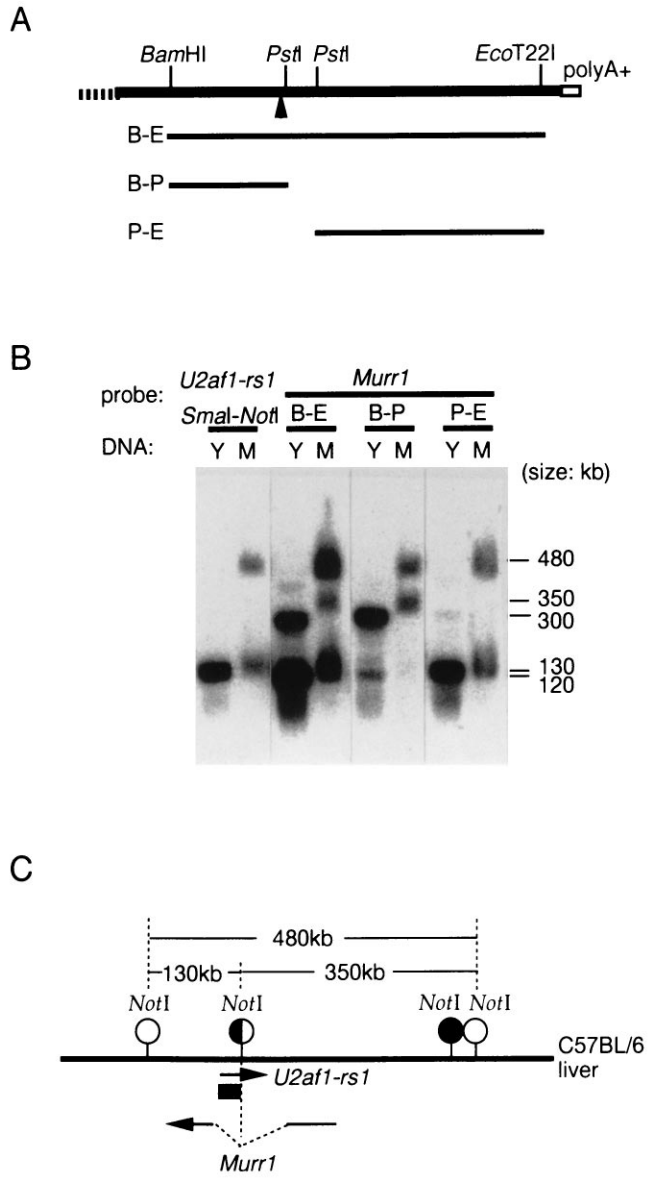

FIG. 2. Mapping of the *U2af1-rs1* gene in an intron of the *Murr1* gene. (A) The cDNA structure of *Murr1* and the relative positions of the probes used for Southern analysis. Fragment B-P (184 bp) is a *Bam*HI-*Pst*I fragment and was used to probe the 5′ part, while fragment P-E (447 bp) is a *Pst*I-*Eco*T22I fragment and was used to probe the 3′ part. The B-E probe (712 bp) is a *Bam*HI-*Eco*T22I fragment that contains almost the whole *Murr1* cDNA region. The position of the intron that contains the *U2af1-rs1* gene (Fig. 3B) is indicated (triangle). (B) Southern analysis of YAC 11/SP2#8 (Y) or chromosomal DNA from the liver cells of female B6 mice (M). Each of the two YAC DNA bands identified by probe B-E was also detected by probe B-P or P-E. (C) The map constructed for the B6 liver DNA. The directions of transcription of both *Murr1* and *U2af1-rs1* genes (arrows), an intron of *Murr1* (dotted line), and the position of the *Sma*I-*Not*I fragment of the *U2af1-rs1* gene (dotted box) are shown. The methylation status of each *Not*I site is indicated as described for Fig. 1B.

RESULTS

Identification of novel genes that are located around the imprinted gene *U2af1-rs1*. To analyze the genome structure of the region around the mouse imprinted gene *U2af1-rs1*, we used genomic DNA clones from YAC and P1 libraries that contained the *U2af1-rs1* gene. When we analyzed the P1 clones, we found a kind of transcript in the insert which was distinct from *U2af1-rs1*. By screening the cDNA library of neonatal B6 mice, we obtained the cDNA clone of interest. A part of this cDNA sequence has been identified as an expressed sequence tag (EST), MUSGS00552, expressed in mouse embryo. A cDNA similar to MUSGS00552 in human found in a database, named ye60f08, is an EST in the fetal liver and spleen. The mouse and human cDNAs had strong homology (87.3% in the sequenced region), and it was suggested that they are homologous genes. We named them *Murr1* (mouse) and *MURR1* (human), for the gene that locates in the mouse *U2af1-rs1* region. These sequences had no significant homology to any other sequence that has been reported so far.

To isolate other genes located around the mouse *U2af1-rs1* gene, we screened the same cDNA library using the whole fragment of YAC clone 11/SP2#8 as a probe (30). We identified four cDNAs, and three were found to be *U2af1-rs1*, *Murr1*, and *Cct4*. We have previously shown that the *Cct4* gene is located on 11/SP2#8 (30). Sequence analysis of the last cDNA, named *Murr2*, revealed that it is a novel mouse gene. It has significant homology to clone HEA02T (91.3% in the sequenced region), which is an EST expressed in human heart.

Physical mapping of these genes was performed with YAC clone 11/SP2#8. *Not*I digests of chromosomal DNA from yeast cells containing 11/SP2#8 or liver cells of female B6 mice were resolved by PFGE, and Southern hybridization analysis was performed using the cDNA fragments as probes (Fig. 1A). The *Not*I-*Eco*RV probe of the *U2af1-rs1* gene cross-hybridized to a 300-kb band of the YAC DNA. This was the same size as one of the two bands detected by the *Murr1* probe. The other was a 120-kb band, the same size as the band detected by the YAC left-end probe (data not shown), while the 280-kb YAC fragment detected by the *Murr2* probe was the same as that detected by the right-end probe (Fig. 1A). Two bands (350 and 480 kb) found in B6 cells by the *U2af1-rs1* or *Cct4* probe were due to the allele-specific status of methylation of the *Not*I sites in the *U2af1-rs1* gene (16, 18, 30). The 350-kb band showed that the *Not*I site, which is found 300 kb away from *U2af1-rs1*, is fully methylated in B6 liver cells. The result obtained with the *Murr1* probe agreed with this finding. The position of *Murr1* in relation to the *U2af1-rs1* gene is described in detail below. The locations of genes on YAC 11/SP2#8 are summarized in Fig. 1B.

An intron of the mouse *Murr1* gene contains the imprinted gene *U2af1-rs1*. To map the mouse *Murr1* gene relative to the *U2af1-rs1* gene, we performed restriction mapping and sequencing (Fig. 2 and 3). Pulsed-field mapping analyses of the *Not*I digests of genomic DNAs which were prepared from yeast cells with 11/SP2#8 and from liver cells of female B6 mice were executed (Fig. 2B). Three kinds of *Murr1* cDNA fragments, B-P (for the 5′ region), P-E (for the 3′ region), and B-E (for the whole region), were used as probes (Fig. 2A). The 5′ and 3′ cDNA probes hybridized with the 300- and 120-kb bands of *Not*I-digested YAC DNA, respectively. The 300-kb *Not*I band was detected with a *Not*I-*Eco*RV probe (downstream region) of *U2af1-rs1* (Fig. 1A), and the 120-kb band was detected with a *Sma*I-*Not*I probe (upstream) (Fig. 2B). Since *U2af1-rs1* has one *Not*I site and no introns, we concluded that the *Murr1* gene has an intron that contains the whole *U2af1-rs1* gene. The results with mouse DNA coincided with those for the YAC clone. The 5′ probe of *Murr1* hybridized to 350- and 480-kb bands of *Not*I-digested B6 DNA, while the 3′ probe hybridized to 130- and 480-kb bands. Two bands detected in mouse liver cells were due to allele-specific methylation of the *Not*I site in the *U2af1-rs1* gene. The bands detected by the whole cDNA fragment probe were those detected by both 5′

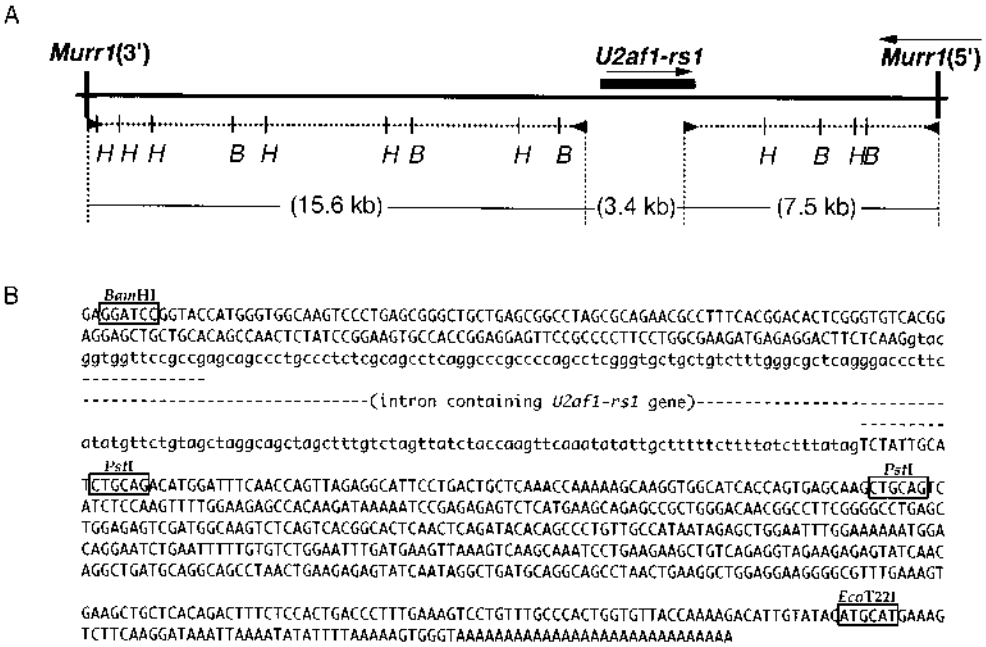

FIG. 3. The structure of the *Murr1* intron that contains the *U2af1-rs1* gene. (A) Restriction map of the intron. The exons (solid boxes) and the directions of transcription (arrows) for each gene, the fragments obtained by LA-PCR (dotted lines), and the positions of primer pairs (arrowheads) are shown. The positions of the *Bam*HI (*B*) and *Hin*dIII (*H*) sites are also indicated. (B) DNA sequences of the exon-intron junction of the *Murr1* gene. The exon and intron sequences are indicated (uppercase and lowercase, respectively). The restriction sites shown in the sequences were used to obtain the probe for pulsed-field mapping in Fig. 2.

and 3′ probes in either YAC or mouse DNA. The result of restriction mapping of *Murr1* gene is summarized in Fig. 2C.

The genomic structure of this region, which is within an intron of *Murr1*, was confirmed by sequencing the exon-intron junctions (Fig. 3). By LA-PCR using two pairs of oligonucleotide primers corresponding to the cDNA sequence of *Murr1* and the genomic sequence of *U2af1-rs1*, we amplified DNA fragments from the P1 clones. Restriction mapping of the amplified DNA fragments showed that the size of this intron of *Murr1* gene is about 26.5 kb (Fig. 3A). As expected from Southern analysis, the transcription of the two genes occurs in opposite directions. Comparison of the cDNA and genomic DNA sequences revealed the exon-intron junctions of *Murr1* in this region (Fig. 3B). This is the first example of an endogenous imprinted gene (*U2af1-rs1*) found in the intron of another gene (*Murr1*).

Chromosomal homology and syntenic relation of the mouse *U2af1-rs1* region. The *U2af1-rs1* gene has been assigned to the proximal region of chromosome 11 by genetic mapping (18, 21). In another report, we showed that the *Cct4* gene is located within about 300 kb of *U2af1-rs1* (30). Since the homolog of *Cct4* was assigned to human chromosome 2 and none of the *U2af1*-related genes were mapped on it (23, 25), there may be a junction of chromosomal homology between the *U2af1-rs1* and the *Cct4* genes (30).

When we analyzed the syntenic relation of this region, we expected to find the feature of the genomic structure of *U2af1-rs1*. We then tried to determine to which chromosome the human *MURR1* gene is assigned. We used the microcell hybrid panel, which is a set of mouse cell lines each containing a single human chromosome (26). Chromosomal DNAs from this panel were analyzed by PCR using a pair of primers for human *MURR1*, and the amplified fragment was observed only in the hybrid cell line that carried human chromosome 2 (Fig. 4A). Furthermore, Southern analysis showed that the mouse *Murr1* cDNA fragment was hybridized with the specific restriction fragments of human genomic DNA, and they were on human chromosome 2, rather than chromosome 5 (Fig. 4B). These results indicated that *MURR1*, the human homolog of the mouse *Murr1* gene, was assigned to chromosome 2. According to the location of the mouse *Murr1*, *Cct4*, and other genes, we concluded that this region is conserved between mouse and human genes (Fig. 4C). However, as described above, none of the *U2af1*-related genes were identified on human chromosome 2. To explain this, we propose that a transposition into this region would result in the formation of the mouse imprinted gene *U2af1-rs1*, as discussed below.

Imprint tests for genes around the mouse *U2af1-rs1* gene. Ten of 18 endogenous imprinted genes so far reported cluster into two domains of the genome (2). If similar mechanisms of genomic imprinting occur in the mouse *U2af1-rs1* region and if colocalization is conserved, some imprinted genes might exist near the *U2af1-rs1* gene. Thus, we tested for genomic imprinting of the genes around *U2af1-rs1.*

As reported before, *U2af1-rs1* is imprinted and the paternal allele is preferentially expressed (16, 18), while *Cct4* is expressed biallelically (30). We examined which allele(s) is expressed by using a polymorphism in the exon sequence of the *Murr1* or *Murr2* gene between two mouse strains, B6 and PWK. The fragments amplified by RT-PCR were subjected to digestion with *Bsp*HI (*Murr1*) or sequencing analysis (*Murr2*). When we analyzed the poly(A)$^+$ RNAs prepared from the neonatal progenies derived by reciprocal backcrosses of two strains, both the B6 and the PWK alleles of *Murr1* and *Murr2* were found to be transcribed in both kinds of F_1 mice (Fig. 5). These results showed that both *Murr1* and *Murr2* are expressed biallelically, and therefore they are not imprinted in neonatal mice. The location of the imprinted gene *U2af1-rs1* within an intron of the biallelically expressed gene *Murr1* suggested that *U2af1-rs1* is isolated from other imprinted genes.

Methylation status of the *U2af1-rs1* region. DNA methylation specific to the silent allele was observed previously in

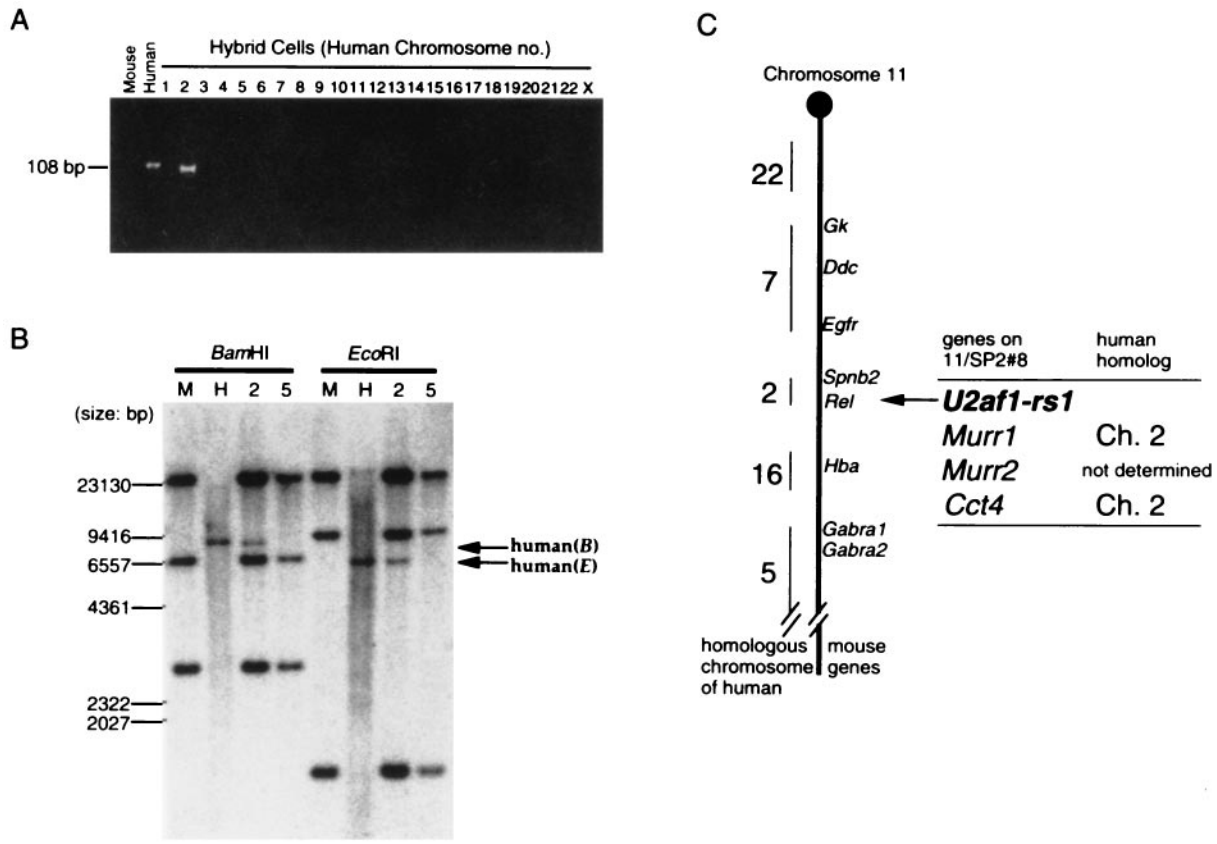

FIG. 4. The chromosomal location of the mouse *Murr1* and *U2af1-rs1* genes on the proximal region of chromosome 11 syntenic to human chromosome 2. (A) Chromosomal assignment of the human *MURR1* gene by PCR analysis. By using a pair of primers specific for the human *MURR1* gene, a 108-bp fragment was amplified by PCR. Lanes Mouse and Human, original mouse and human cells used for constructing this hybrid cell panel. (B) Chromosomal assignment of the human gene homologous to the mouse *Murr1* gene. Five micrograms of chromosomal DNA from hybrid cells containing human chromosome 2 (lanes 2) or 5 (lanes 5) was subjected to Southern analysis. Lanes M and H, the same mouse and human cells as in panel A. The probe was the mouse *Murr1* cDNA fragment (B-E in Fig. 2A). The bands specific to human genes are noted (arrow). (C) A genetic map of the proximal region of mouse chromosome 11. The human homologs of *Murr1* and *Cct4* in 11/SP2#8 were assigned to chromosome 2 (Ch. 2) (panels A and B) (23). Since the mouse *U2af1-rs1* gene is mapped to the region of conserved synteny to human chromosome 2, and since no *U2af1*-related genes were found on it, there are no corresponding counterparts of mouse imprinted gene *U2af1-rs1* in human.

imprinted genes (33). In the case of *U2af1-rs1*, the silent maternal allele is hypermethylated (16, 18). Therefore, by analyzing the methylation status of the region around the mouse *U2af1-rs1* gene, we tried to estimate the chromosomal domain in which the genomic imprinting mechanism operates.

We constructed a restriction map of the region around the *U2af1-rs1* gene by Southern analyses of 11/SP2#8 and genomic DNA from the liver of adult female B6 mice (Fig. 6). Restriction endonucleases sensitive to cytosine methylation were used to analyze the methylation status of this region. The YAC DNA was used as a control for negative methylation. Long-range restriction mapping by PFGE revealed that the *Not*I-*Eco*RV probe of *U2af1-rs1* cross-hybridized with two bands of 30 and 180 kb in the *Not*I- and *Bss*HII-digested B6 DNA and two bands of 20 and 165 kb in the *Not*I- and *Sac*II-digested B6 DNA (Fig. 6A). The larger fragments of the two digests were deduced to be from the allele with the methylated *Not*I site of *U2af1-rs1*, and the smaller ones were deduced to be from the allele with the unmethylated site. The smaller ones were the same size as the YAC bands. These results showed that both *Bss*HII and *Sac*II sites were unmethylated, each of which is about 20 or 30 kb away from the *U2af1-rs1* gene.

We further examined the methylation status in the upstream and downstream regions of *U2af1-rs1*, which are included in an intron of the *Murr1* gene. Southern blot analyses demonstrated that the methylation pattern of the *U2af1-rs1* gene was limited to within the intron of *Murr1* (Fig. 6C). For instance, both paternal and maternal alleles of the *Bsa*AI site (site 1) located about 5 kb upstream of the transcription initiation site were completely methylated. In the upstream region, the most distant site that is partially methylated, like the coding region, was the *Hpa*II site located about 2.5 kb upstream of the initiation site (site 2/*Hpa*II). The *Hha*I site about 1 kb downstream from the poly(A)$^+$ signal was fully methylated. The results of the restriction mapping analyses are summarized in Fig. 6B and D.

Finally, we tested the allele specificity of partially methylated sites. The PCR-based method was applied to chromosomal DNA from liver cells of two strains obtained by reciprocal crosses between B6 and PWK. We found that the methylation status of site 2/*Hpa*II was independent from parental origin while that of the *Sma*I/*Hpa*II site (0.2 kb upstream) was specific to the maternal allele (Fig. 7B). This allelism of the *Sma*I/*Hpa*II site was consistent with the result of Southern analysis using restriction fragment length polymorphism (16). The methylation of site 3/*Hpa*II was also particular to the maternal allele (data not shown). These results suggested that the region with allele-specific methylation status is limited to just around *U2af1-rs1*. This restricted area of the methylation pattern corresponds well to the biallelic pattern of *Murr1* gene expression.

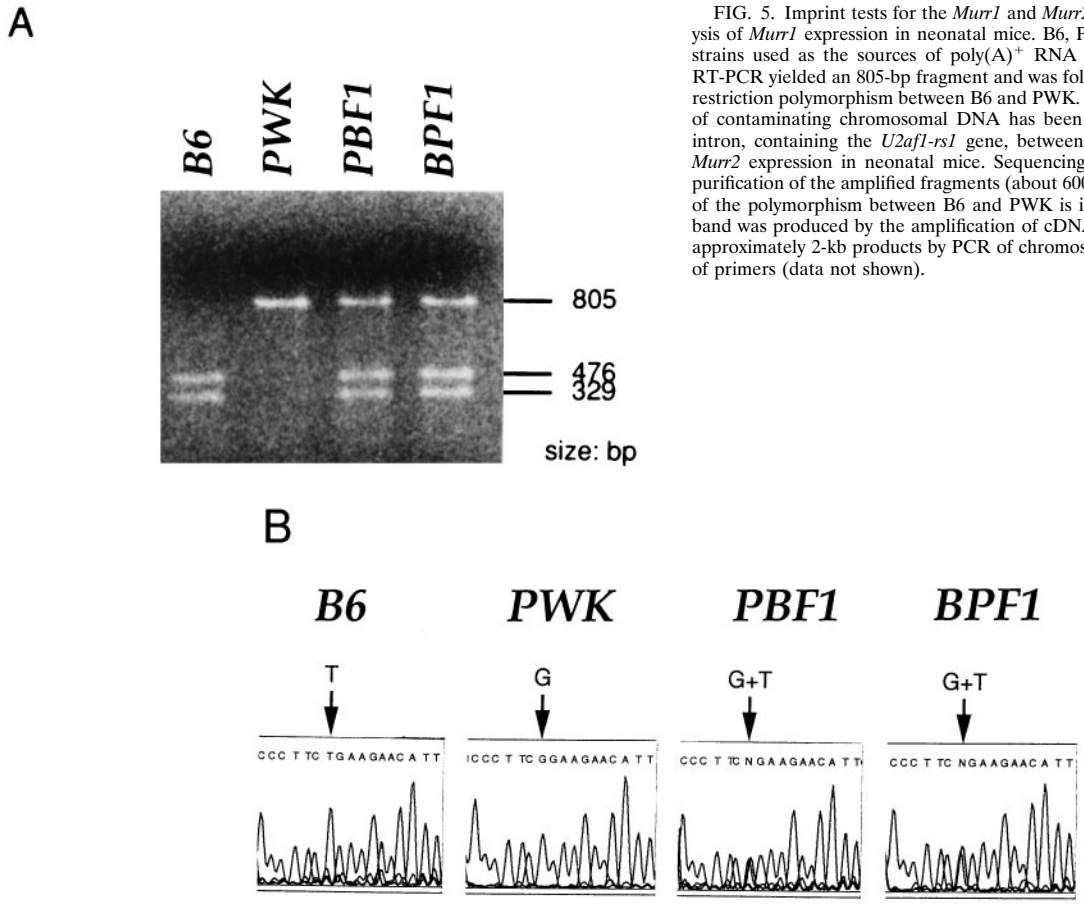

FIG. 5. Imprint tests for the *Murr1* and *Murr2* genes by RT-PCR. (A) Analysis of *Murr1* expression in neonatal mice. B6, PWK, PBF1, and BPF1, mouse strains used as the sources of poly(A)$^+$ RNA (see Materials and Methods). RT-PCR yielded an 805-bp fragment and was followed by *Bsp*HI digestion for a restriction polymorphism between B6 and PWK. The possibility of amplification of contaminating chromosomal DNA has been excluded because there is an intron, containing the *U2af1-rs1* gene, between two primers. (B) Analysis of *Murr2* expression in neonatal mice. Sequencing analysis was performed after purification of the amplified fragments (about 600 bp) by RT-PCR. The position of the polymorphism between B6 and PWK is indicated (arrows). The 600-bp band was produced by the amplification of cDNA, not chromosomal DNA, for approximately 2-kb products by PCR of chromosomal DNA with the same pair of primers (data not shown).

DISCUSSION

In this study, we elucidated the genome structure of the mouse imprinted gene *U2af1-rs1*, the imprinting status of genes around *U2af1-rs1*, and the methylation status of this chromosomal region. First, we identified mouse genes located around the imprinted gene *U2af1-rs1*. The structure of the genomic DNA revealed that one of them, named *Murr1*, contains the whole *U2af1-rs1* gene within one of its introns. The human homolog of *Murr1* is mapped to chromosome 2, as are some of the other genes on the proximal region of mouse chromosome 11. Second, we tested *Murr1* and *Murr2* for imprinting. Biallelic expression of the *Murr1* gene suggests that the genome structure around the *U2af1-rs1* gene is distinct from the two chromosomal domains where several imprinted genes cluster. Third, the methylation status characteristic of imprinted genes was found to be limited to just around the *U2af1-rs1* gene. These results suggested that the mouse *U2af1-rs1* gene has a neomorphic origin and that other endogenous imprinted genes might arise by transposition.

The origin of the *U2af1-rs1* gene as a transposon. We showed that the mouse *U2af1-rs1* gene is located on the region of conserved synteny to human chromosome 2 (Fig. 4). The human homologs of the *Murr1* and *Cct4* genes were assigned to chromosome 2 (Fig. 4) (23). Mouse *Murr1* has an approximately 26.5-kb intron that contains the whole of *U2af1-rs1* (Fig. 2 and 3), while *Cct4* is located within 300 kb of *U2af1-rs1* (30). *Spnb2* (beta-spectrin 2 gene) and *Rel* (reticuloendotheliosis oncogene), which are located in the proximal region of mouse chromosome 11, are other examples of genes whose human homologs are also mapped to chromosome 2 (data obtained from the Mouse Genome Database [MGD] at Jackson Laboratory and from the Genome Data Base [GDB] at Johns Hopkins University).

However, none of the three *U2af1*-related genes were assigned to human chromosome 2 (25). Furthermore, Southern analyses of the microcell hybrid panel suggested that there are no *U2af1*-related genes on human chromosome 2 (25, 29). The discrepancy of chromosomal homology around the mouse *U2af1-rs1* gene suggested that its origin is distinct from the genes around it. It is likely that transposition into an intron of the *Murr1* gene has resulted in the formation of *U2af1-rs1*. The genome structure of *U2af1-rs2*, another *U2af1*-related gene in mouse on chromosome X, supports this idea. *U2af1-rs1* is intronless, while the *U2af1-rs2* gene seems to have introns, as determined by Southern analysis (28). We hypothesize that the retrotransposition of *U2af1-rs2* on the X chromosome could form the *U2af1-rs1* gene. This transposition must have occurred after mice and humans diverged, because there are no *U2af1*-related genes on human chromosome 2.

It is true that the human *U2AF1-RS1* gene on chromosome 5 had been regarded as the homolog of the mouse imprinted gene *U2af1-rs1* (21, 25). However, we should amend this idea because it is inconsistent with some apparent results. At first, in either nucleotide or amino acid sequence, human *U2AF1-RS1* appeared to be more similar to mouse *U2af1-rs2* on chromosome X than to *U2af1-rs1* (25). In this study, we showed that the mouse *U2af1-rs1* gene was mapped within an intron of the *Murr1* gene, on the region with conserved synteny to human

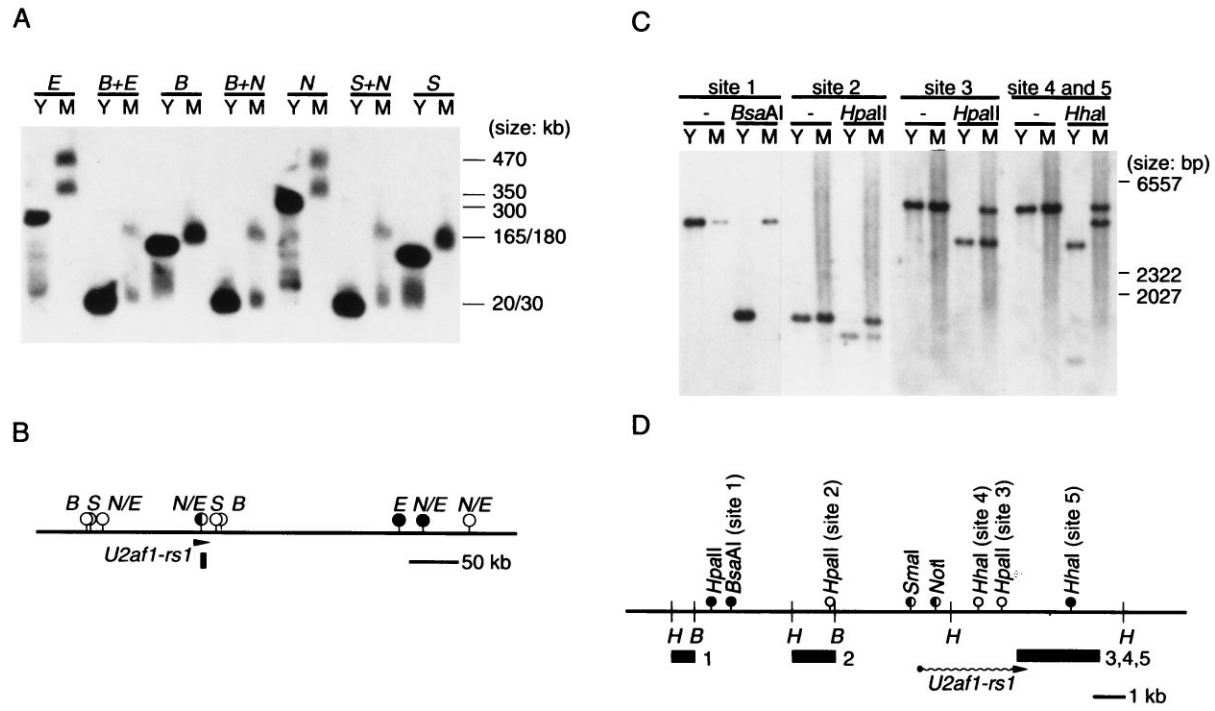

FIG. 6. Methylation status of the *U2af1-rs1* region. (A) Long-range methylation analysis by PFGE using restriction endonucleases sensitive to DNA methylation. Y and M, YAC 11/SP2#8 and female B6 mouse liver DNA, respectively; *B*, *Bss*HII; *E*, *Eag*I; *N*, *Not*I; and *S*, *Sac*II. The probe used was the *Not*I-*Eco*RV fragment of *U2af1-rs1* (striped box in Fig. 1B). (B) Summary of the long-range methylation analysis of female B6 liver DNA. Restriction sites which were unmethylated, methylated, and allele-specific methylated, (open, solid, and half-solid lollipops, respectively) are shown. (C) Methylation status of the flanking regions of the mouse *U2af1-rs1* gene. 11/SP2#8 (Y) or B6 (M) DNAs were digested with *Hin*dIII (sites 1 and 3 to 5) or *Bam*HI and *Hin*dIII (site 2) and subjected to Southern hybridization analyses. The restriction enzymes indicated are sensitive to methylation. (D) Summary of the methylation analysis of female B6 mouse liver DNA around the *U2af1-rs1* gene. The lollipop symbols are the same as for panel B, and the hatched ones represent partially methylated sites. Transcription of *U2af1-rs1* (wavy line) and the initiation site and poly(A)$^+$ signal (solid circle and arrowhead, respectively) are shown. The probes used for Southern analyses (solid boxes) and the sites analyzed by these probes (numbers) and the positions of the *Bam*HI (*B*) and *Hin*dIII (*H*) sites are also indicated.

chromosome 2. To account for all results reported to date, we concluded that the human *U2AF1-RS1* gene is not the counterpart of mouse *U2af1-rs1*. This view could be consistent with the absence of imprinting of human *U2AF1-RS1* (24, 32).

The unique genome structure of *U2af1-rs1* as an imprinted gene. Clustering into chromosomal domains was thought of as one of the characteristic features of endogenous imprinted genes (2). This feature implied that the mechanism causing genomic imprinting would function over long chromosomal regions. However, the mouse *U2af1-rs1* gene does not appear to be clustered. We showed that neither the *Murr1* nor the *Murr2* gene is imprinted (Fig. 5) and that the *U2af1-rs1* gene is located in an intron of the *Murr1* gene (Fig. 2 and 3). These results suggested that the chromosomal domain under the control of genomic imprinting is limited to the region just around the *U2af1-rs1* gene. This idea is supported by the limited area of allele-specific methylation around the *U2af1-rs1* gene (Fig. 6 and 7). There may be boundaries of the chromosomal domains near *U2af1-rs1*, one that is imprinted and another that is not imprinted. Since these boundaries seemed to correlate with the discrepancy of chromosomal homology in the *Murr1* intron (Fig. 4), the imprinting mechanism of *U2af1-rs1* may be related to its origin as a transposon.

Among the endogenous imprinted genes reported so far, the mouse *Ins1* (insulin 1) gene may have features in common with *U2af1-rs1*, as it is an imprinted transposon (10). The *Ins1* gene on chromosome 19 is thought to originate by retrotransposition of the other insulin gene, *Ins2*, on chromosome 7 (39). Only one insulin gene, which is homologous to the mouse *Ins2* gene, was found on human chromosome 11, and therefore *Ins1* must have formed after mice and humans diverged, like the *U2af1-rs1* gene.

Mechanisms of the genomic imprinting of neomorphic genes. As described above, we hypothesize that the *U2af1-rs1* gene has arisen by retrotransposition. This suggests that the mechanism of the imprinting of *U2af1-rs1* may be related to that of transgene imprinting. It has been shown that some transgenes exhibit allele-specific expression and methylation patterns dependent on the parental lineage (13, 34, 36, 45). As a result of the transposition into an intron of *Murr1*, the neomorphic *U2af1-rs1* gene behaves like an imprinted transgene. Although we are not yet sure how the other endogenous imprinted genes originated, it is possible that some of them were created by transposition. If retrotransposition is a common mechanism for the formation of imprinted genes, they may tend to have few and small introns (14, 19).

Since the mouse *U2af1-rs2* gene, the hypothetical origin of *U2af1-rs1*, was not imprinted (47), the original structure before transposition is not thought to contain the complete *cis*-acting structure for imprinting in its DNA sequences. This suggests that whether a transposon becomes imprinted depends on its position of insertion and/or its modifications. The unique repeats found in the 5′ untranslated region of the mouse *U2af1-rs1* gene (16) may represent the modification necessary for a gene to be imprinted. This idea is supported by the absence of imprinting of *U2AF1-RS1*, which is one of the *U2af1*-related genes on human chromosome 5 and does not contain any repetitive sequences (24, 32). Furthermore, nuclease-hyper-

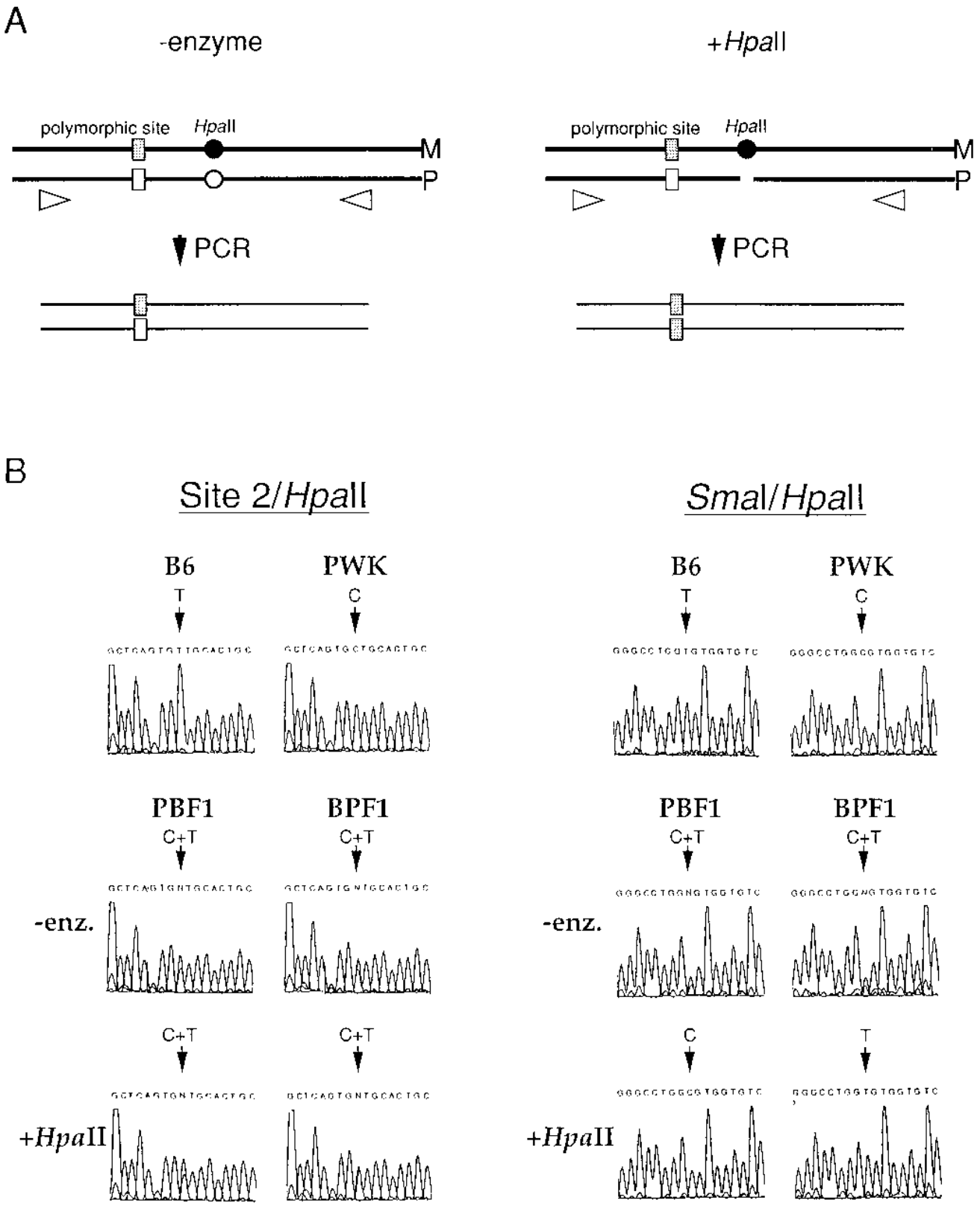

FIG. 7. Allelic analysis of methylation. (A) Summary of the PCR-based method to analyze the allelism of methylation. As an example, the case that the *Hpa*II site is methylated specifically to its maternal allele is shown. Methylated and unmethylated *Hpa*II sites (solid and open circles, respectively) are indicated. After discovering polymorphism (boxes) between two strains of mice near the *Hpa*II site of interest, chromosomal DNA of F_1 mice derived from the reciprocal crosses of two strains were amplified by PCR. A pair of primers (triangles) were placed to include both the polymorphic site and the *Hpa*II site in the PCR products. If the *Hpa*II digestion is prior to PCR, only the chromosomal DNA with the methylated *Hpa*II site will be used for the amplification. Sequencing analysis of the PCR products is carried out to determine which allele(s) is amplified. (B) Results of the allelism test of methylation of site 2/*Hpa*II and *Sma*I/*Hpa*II. The four mouse strains are the same as for Fig. 5. For *Hpa*II-digested samples (+*Hpa*II), the polymorphic nucleotides from both parents were observed in site *2/HpaII*, whereas only the maternal nucleotides were observed in the *Sma*I/*Hpa*II site. enz., enzyme.

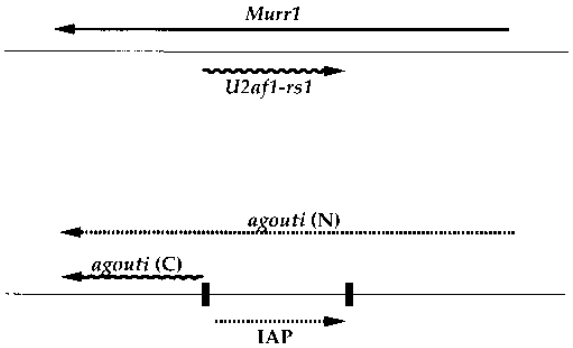

FIG. 8. Similarities in the genome structure of the *U2af1-rs1* region and the dominant alleles of the *agouti* mutation (7, 27). The transcripts in each region (arrows) are indicated (transcripts of allele-specific [wavy lines] or biallelic [solid lines] expression). The expression patterns of transcripts represented by the dotted line were not determined. The phenotype dependent on the parental lineage of the dominant *agouti* alleles is due to ectopic transcription from the cryptic promoter [*agouti* (C)] in the long terminal repeat (solid boxes) of IAP, rather than that from the native *agouti* promoter [*agouti* (N)].

sensitive sites of chromatin specific to transcribed or paternal alleles were organized just around the repetitive sequences of the mouse *U2af1-rs1* gene (29, 38). Repetitive sequences observed in some imprinted genes have been discussed in relation to the mechanisms of genomic imprinting (31).

We might obtain some more information about the mechanisms of transposon imprinting from the features of the mouse *U2af1-rs1* genome structure itself. This gene is transcribed in the direction opposite to that of the *Murr1* gene (Fig. 2 and 3). If a transposon is inserted into a region with transcription in the opposite direction, it may have a chance to be imprinted. It has been reported that some dominant *agouti* alleles in mice contain a retrotransposon-like element, IAP (intracisternal A-particle) (7, 27). Each IAP insertion found in three different alleles is oriented in the direction opposite to the *agouti* transcription. The expression of the *agouti* phenotypes of these alleles is dependent on the parental lineage, and the common mechanism for the imprinting of neomorphic genes would function in the case of the mouse *U2af1-rs1* gene (Fig. 8). It is difficult to compare the two examples directly, but it is possible that the transcription of the IAPs, which corresponds to *U2af1-rs1*, is imprinted, too. This is supported by the fact that the methylation status of IAPs is dependent on their parental origin (35).

Further analyses may reveal whether the other endogenous imprinted genes are neomorphic and whether they have mechanisms related to that of *U2af1-rs1*. In addition, the mouse *U2af1-rs1* region could help in the analysis of the mechanism of genomic imprinting, since the *cis*-acting region for imprinting would be restricted in the *Murr1* intron. Analysis of this region may reveal a simple model for genomic imprinting and provide clues to the mechanisms of allele-specific regulation of gene expression.

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REFERENCES

1. **Ariel, M., E. Robinson, J. R. McCarrey, and H. Cedar.** 1995. Gamete-specific methylation correlates with imprinting of the murine *Xist* gene. Nat. Genet. **9:**312–315.
2. **Barlow, D. P.** 1995. Gametic imprinting in mammals. Science **270:**1610–1613.
3. **Barnes, W. M.** 1994. PCR amplification of up to 35-kb DNA with high fidelity and high yield from lambda bacteriophage templates. Proc. Natl. Acad. Sci. USA **91:**2216–2220.
4. **Bartolomei, M. S., A. L. Webber, M. E. Brunkow, and S. M. Tilghman.** 1993. Epigenetic mechanisms underlying the imprinting of the mouse *H19* gene. Genes Dev. **7:**1663–1673.
5. **Brandeis, C. V., T. Kafri, M. Ariel, J. R. Chaillet, J. R. McCarrey, A. Razin, and H. Cedar.** 1993. The ontogeny of allele-specific methylation associated with imprinted genes in the mouse. EMBO J. **12:**3669–3677.
6. **Cheng, S., C. Fockler, W. M. Barnes, and R. Higuchi.** 1994. Effective amplification of long targets from cloned inserts and human genomic DNA. Proc. Natl. Acad. Sci. USA **91:**5695–5699.
7. **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.
8. **Feinberg, A. P., and B. A. Vogelstein.** 1983. A technique for radiolabelling DNA restriction fragments to high specific activity. Anal. Biochem. **132:**6–13.
9. **Ferguson, S. A., H. Sasaki, B. M. Cattanach, and M. A. Surani.** 1993. Parental-origin-specific modification of the mouse *H19* gene. Nature (London) **362:**751–755.
10. **Giddings, S. J., C. D. King, K. W. Harman, J. F. Flood, and L. R. Carnaghi.** 1994. Allele specific inactivation of insulin 1 and 2, in the mouse yolk sac, indicates imprinting. Nat. Genet. **6:**310–313.
11. **Guillemot, F., T. Caspary, S. M. Tilghman, N. G. Copeland, D. J. Gilbert, N. A. Jenkins, D. J. Anderson, A. L. Joyner, J. Rossant, and A. Nagy.** 1995. Genomic imprinting of *Mash2*, a mouse gene required for trophoblast development. Nat. Genet. **9:**235–242.
12. **Guillemot, F., L.-C. Lo, J. E. Johnson, A. Auerbach, D. J. Anderson, and A. L. Joyner.** 1993. Mammalian *achaete-scute* homolog 1 is required for the early development of olfactory and autonomic neurons. Cell **75:**463–476.
13. **Hadchouel, M., H. Farza, D. Simon, P. Tiollais, and C. Pourcel.** 1987. Maternal inhibition of hepatitis B surface antigen gene expression in transgenic mice correlates with *de novo* methylation. Nature (London) **329:**454–456.
14. **Haig, D.** 1996. Do imprinted genes have few and small introns? BioEssays **18:**351–353.
15. **Hatada, I., Y. Hayashizaki, S. Hirotsune, H. Komatsubara, and T. Mukai.** 1991. A genome scanning method for higher organisms using restriction sites as landmarks. Proc. Natl. Acad. Sci. USA **88:**9523–9527.
16. **Hatada, I., K. Kitagawa, T. Yamaoka, X. Wang, Y. Arai, K. Hashido, S. Ohishi, J. Masuda, J. Ogata, and T. Mukai.** 1995. Allele-specific methylation and expression of an imprinted *U2af1-rs1* (*SP2*) gene. Nucleic Acids Res. **23:**36–41.
17. **Hatada, I., and T. Mukai.** 1995. Genomic imprinting of $p57^{KIP2}$, a cyclin-dependent kinase inhibitor, in mouse. Nat. Genet. **11:**204–206.
18. **Hatada, I., T. Sugama, and T. Mukai.** 1993. A new imprinted gene cloned by a methylation-sensitive genome scanning method. Nucleic Acids Res. **21:**5577–5582.
19. **Hurst, L. D., G. McVean, and T. Moore.** 1996. Imprinted genes have few and small introns. Nat. Genet. **12:**234–237.
20. **Joslyn, G., M. Carlson, A. Thliveris, H. Albertsen, L. Gelbert, W. Samowitz, J. Groden, J. Stevens, L. Spirio, M. Robertson, L. Sargeant, K. Krapcho, E. Wolff, R. Burt, J. P. Hughes, J. Warrington, J. McPherson, J. Wasmuth, D. LePaslier, H. Abderrahim, D. Cohen, M. Leppert, and R. White.** 1991. Identification of deletion mutations and three new genes at the familial polyposis locus. Cell **66:**601–613.
21. **Kalcheva, I., C. Plass, S. Sait, R. Eddy, T. Shows, C. D. Watkins, S. Camper, H. Shibata, T. Ueda, N. Takagi, Y. Hayashizaki, and V. Chapman.** 1995. Comparative mapping of the imprinted *U2afbpL* gene on mouse chromosome 11 and human chromosome 5. Cytogenet. Cell Genet. **68:**19–24.
22. **Kay, G. F., G. D. Penny, D. Patel, A. Ashworth, N. Brockdorff, and S. Rastan.** 1993. Expression of *Xist* during mouse development suggests a role in the initiation of X chromosome inactivation. Cell **72:**171–182.
23. **Khan, A. S., A. S. Wilcox, M. H. Polymeropoulos, J. A. Hopkins, T. J. Stevens, M. Robinson, A. K. Orpana, and J. M. Sikela.** 1992. Single pass sequencing and physical and genetic mapping of human brain cDNAs. Nat. Genet. **2:**180–185.
24. **Kitagawa, K., I. Hatada, I. Kondo, and T. Mukai.** Submitted for publication.
25. **Kitagawa, K., X. Wang, I. Hatada, T. Yamaoka, H. Nojima, J. Inazawa, T. Abe, K. Mitsuya, M. Oshimura, A. Murata, M. Monden, and T. Mukai.** 1995. Isolation and mapping of human homologues of an imprinted mouse gene *U2af1-rs1*. Genomics **30:**257–263.
26. **Koi, M., M. Shimizu, H. Morita, H. Yamada, and M. Oshimura.** 1989. Construction of mouse A9 clones containing a single human chromosome tagged with neomycin-resistance gene via microcell fusion. Jpn. J. Cancer Res. **80:**413–418.
27. **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 (A^{iapy}) is correlated with methylation state and is influenced by parental lineage. Genes Dev. **8:**1463–1472.
28. **Mukai, T.** Unpublished data.
29. **Nabetani, A.** Unpublished data.
30. **Nabetani, A., I. Hatada, H. Morisaki, and T. Mukai.** 1996. Chromosome assignment and imprinting test for the mouse delta subunit of the cytosolic chaperonin containing TCP-1 (*Cct4*) gene to proximal chromosome 11. Genomics **34:**246–249.
31. **Neumann, B., P. Kubicka, and D. P. Barlow.** 1995. Characteristics of imprinted genes. Nat. Genet. **9:**12–13.
32. **Pearsall, R. S., H. Shibata, A. Brozowska, K. Yoshino, K. Okuda, P. J. deJong, C. Plass, V. M. Chapman, Y. Hayashizaki, and W. A. Held.** 1996. Absence of imprinting in *U2AFBPL*, a human homologue of the imprinted mouse gene *U2afbp-rs*. Biochem. Biophys. Res. Commun. **222:**171–177.
33. **Razin, A., and H. Cedar.** 1994. DNA methylation and genomic imprinting. Cell **77:**473–476.
34. **Reik, W., A. Collick, M. L. Norris, S. C. Barton, and M. A. Surani.** 1987. Genomic imprinting determines methylation of parental alleles in transgenic mice. Nature (London) **328:**248–251.
35. **Sanford, J. P., H. J. Clark, V. M. Chapman, and J. Rossant.** 1987. Differences in DNA methylation during oogenesis and spermatogenesis and their persistence during early embryogenesis in the mouse. Genes Dev. **1:**1039–1046.
36. **Sapienza, C., A. C. Peterson, J. Rossant, and R. Balling.** 1987. Degree of methylation of transgenes is dependent on gamete of origin. Nature (London) **328:**251–254.
37. **Sealy, P. G., P. A. Whittaker, and E. M. Southern.** 1985. Removal of repeated sequences from hybridization probes. Nucleic Acids Res. **13:**1905–1922.
38. **Shibata, H., K. Yoshino, S. Sunahara, Y. Gondo, M. Katsuki, T. Ueda, M. Kamiya, M. Muramatsu, Y. Murakami, I. Kalcheva, C. Plass, V. M. Chapman, and Y. Hayashizaki.** 1996. Inactive allele-specific methylation and chromatin structure of the imprinted gene *U2af1-rs1* on mouse chromosome 11. Genomics **35:**248–252.
39. **Soares, M. B., E. Schon, A. Henderson, S. K. Karathanasis, R. Cate, S. Zeitlin, J. Chirgwin, and A. Efstratiadis.** 1985. RNA-mediated gene duplication: the rat preproinsulin I gene is a functional retroposon. Mol. Cell. Biol. **5:**2090–2103.
40. **Solter, D.** 1988. Differential imprinting and expression of maternal and paternal genomes. Annu. Rev. Genet. **22:**127–146.
41. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:**503–517.
42. **Stoger, R., P. Kubicka, C. G. Liu, T. Kafri, A. Razin, H. Cedar, and D. P. Barlow.** 1993. Maternal-specific methylation of the imprinted mouse *Igf2r* locus identifies the expressed locus as carrying the imprinting signal. Cell **73:**61–71.
43. **Strauss, W. M., E. Jaenisch, and R. Jaenisch.** 1992. A strategy for rapid production and screening of yeast artificial chromosome libraries. Mammal. Genome **1:**150–157.
44. **Sutcliffe, J. S., M. Nakao, S. Christian, K. H. Orstavik, N. Tommerup, D. H. Ledbetter, and A. L. Beaudet.** 1994. Deletions of a differentially methylated CpG island at the *SNRPN* gene define a putative imprinting control region. Nat. Genet. **8:**52–58.
45. **Swain, J. L., T. A. Stewart, and P. Leder.** 1987. Parental legacy determines methylation and expression of an autosomal transgene: a molecular mechanism for parental imprinting. Cell **50:**719–727.
46. **Tremblay, K. D., J. R. Saam, R. S. Ingram, S. M. Tilghman, and M. S. Bartolomei.** 1995. A paternal-specific methylation imprint marks the alleles of the mouse *H19* gene. Nat. Genet. **9:**407–413.
47. **Yamaoka, T., I. Hatada, K. Kitagawa, X. Wang, and T. Mukai.** 1995. Cloning and mapping of the *U2af1-rs2* gene with a high transmission distortion in interspecific backcross progeny. Genomics **27:**337–340.
48. **Zemel, S., M. S. Bartolomei, and S. M. Tilghman.** 1992. Physical linkage of two mammalian imprinted genes, *H19* and insulin-like growth factor 2. Nat. Genet. **2:**61–65.
49. **Zuccotti, M., and M. Monk.** 1995. Methylation of the mouse *Xist* gene in sperm and eggs correlates with imprinted *Xist* expression and paternal X-inactivation. Nat. Genet. **9:**316–320.