Identification of a novel isoform of *Murr1* transcript, *U2mu*, which is transcribed from the portions of two closely located but oppositely oriented genes

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(Received 7 June 2002, accepted 2 September 2002)

Here we identified a novel transcript in mouse that is transcribed from the portions of two independent genes, *U2af1-rs1* and *Murr1*, and we designated it *U2mu*. The *U2af1-rs1* gene is located in the intron and transcribed in the opposite direction from the *Murr1* gene on the proximal region of mouse chromosome 11. The *U2mu* cDNA sequence is derived from three genomic regions – an intron of the *Murr1* gene, an antisense sequence of *U2af1-rs1* gene, and the last exon of *Murr1* gene – in the order of 5' to 3'. The *U2mu* transcript of 2.8 kb is expressed ubiquitously in adult mice. It is transcribed biallelically, and is not imprinted, in neonatal and adult mice.

The *U2af1-rs1* gene is located on the proximal region of mouse chromosome 11 and within an intron of the *Murr1* gene (Nabetani et al., 1997). The *U2af1-rs1* gene has no intron and is a mouse homologue of a human gene, *U2AF1-RS1*, which encodes a 51kDa protein of the U2AF family, an essential component of a mammalian splicing complex (Tronchere et al., 1997). This gene is transcribed exclusively from the paternal allele in all the adult tissues that have been analyzed so far (Hatada et al., 1995; Hatada et al., 1993; unpublished results). This monoallelic and parent-specific mode of expression is known as genomic imprinting (Solter et al., 1988). The *Murr1* gene is reported not to be imprinted, that is, it is expressed biallelically in neonatal mice (Nabetani et al., 1997). These two genes are transcribed in the opposite directions (Fig. 1).

We have attempted to identify new genes in this genomic region because imprinted genes tend to be clustered and to affect each other's allelic expression, but *U2af1-rs1* is the only imprinted gene in this genomic region so far analyzed. A BLAST search was performed using the sequence of this genomic region as a query against an expressed sequence tags (ESTs) database at the NCBI Web site, (<http://www.ncbi.nlm.nih.gov/dbEST/index.html>). We found a 243 bp mouse 5'-EST sequence (GenBank accession No. W11838) that consists of an antisense sequence (87 bp) of the *U2af1-rs1* gene and a sense sequence (156 bp) of the *Murr1* gene in the 5' and 3' portions, respectively. We purchased the EST clone (Clone ID; 330049, ATCC No. 587162) from American Type Cul-

ture Collection and sequenced the cDNA insert of 675 bp. The comparison of the sequence with the sequences of *U2af1-rs1* and *Murr1* cDNAs (accession Nos. D17407 and D85430, respectively) revealed that the 5' sequence (87 bp) and the 3' sequence (588 bp) were identical with the antisense sequence of *U2af1-rs1* cDNA and the sense sequence of 3' end of *Murr1* cDNA, respectively (Fig. 1). Therefore, we designated the transcript corresponding to this cDNA *U2mu*.

A mouse adult liver cDNA library (Life Technologies Co.) was further screened for *U2mu* cDNA clones using two different probes. One (probe MU) was derived from *Murr1* cDNA; the other (probe U2) was derived from *U2af1-rs1* cDNA. Only one cDNA clone was found to be positive for both probes in the screening of 8×10^6 clones. Sequencing of this cDNA clone revealed that the cDNA insert is 1459 bp long, excluding the polyA tail, (accession No. AB076722) and consists of sequences derived from three portions of the genome, that is, the intron of the *Murr1* gene (679 bp), the antisense sequence of the *U2af1-rs1* gene (204 bp); and the last exon of the *Murr1* gene (576 bp) (Figs. 1, 2). The largest ORF in the sequence encodes a putative protein of 243 amino acids and shares its 3' half portion (encoding 114 amino acids) with the ORF of *Murr1* mRNA (Fig. 2). No sequence homologous to the protein was found by the BLAST search against the protein databases. The existence of *U2mu* mRNA was confirmed by RT-PCR of mouse adult liver RNAs with the primer pairs unk-189/UMU-SR and UMUF/UMUR (Fig. 1) (data not shown).

Using Northern blotting, we assessed the full length and the tissue distribution of *U2mu* mRNA as follows: a premade Mouse Multiple Tissue Northern (MTNR) Blot

Edited by Toshihiko Shiroishi

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Fig. 1. Genomic structure of *Murr1* and *U2af1-rs1* genes. The *U2af1-rs1* gene and the exons of the *Murr1* gene are shown as boxes on the genomic DNA along with the *Murr1* and *U2mu* cDNAs. *Murr1* and *U2mu* are transcribed rightward, and *U2af1-rs1* leftward. Primers used for PCR (see text) are indicated by arrowheads above and below the cDNAs.

(Clontech Co.) was probed with the PCR fragment synthesized with primers unk2 and UMUN (Fig. 1). The probe recognized RNAs of approximately 2.8 kb in all the tissues (Fig. 3a). This result indicates that the *U2mu* transcript was expressed almost ubiquitously, although at a very low level. The main tissues expressing this transcript were brain, lung, liver, and kidney. When the same blot was reprobed with the *Murr1* cDNA probe, 1 kb RNAs were detected at high levels in all the tissues examined with a somewhat different distribution from *U2mu* transcripts. *Murr1* was expressed abundantly in heart, brain, liver, kidney, and testis (Fig. 3b).

The allelic expression of the *U2mu* transcript was analyzed because its antisense gene, *U2af1-rs1*, is imprinted in all the tissues so far examined (Hatada et al., 1995; Hatada et al., 1993; Wang et al., unpublished data). We used a polymorphism in the *BspHI* site between the two mouse strains C57BL/6 (B6) and PWK at position #1031 in the cDNA sequence (Fig. 2). PolyA RNAs were prepared from various tissues of adult F1 progeny obtained by reciprocal crosses between B6 and PWK. RT-PCR with primers UMUF and UMUR2 (Fig. 2) yielded a 531 bp fragment that was suggested to *BspHI* digestion. Both B6 and PWK alleles were found to be expressed in all the tissues examined (Fig. 4). Biallelic expression was also observed in neonatal mice (data not shown). This biallelic expression of *U2mu* transcript suggests that this transcript may not be involved in paternal allele-specific expression of the *U2af1-rs1* gene because antisense transcripts, which have been reported to be involved in the imprinting of their counterpart genes, show reciprocal monoallelic expression (Lyle et al., 2000; Chamberlain et al., 2001).

We hypothesize that the mouse *U2af1-rs1* gene was formed by retro-transposition of its ancestral gene on the

X chromosome into the intron of the ancestral *Murr1* gene in mice arose after mice diverged from humans, because the gene is an intron-less gene and its human homolog has not been identified in the intron of the human *MURR1* gene (Nabetani et al., 1997). This fact indicates that the *U2mu* transcript must have been formed after the divergence between mice and humans via the combination of two sequences, the newly formed sequence, *U2af1-rs1* and the old sequence, *Murr1*. A full-length cDNA clone could not be isolated in spite of extensive screening of the mouse cDNA library, probably due to the very low level of expression of the gene. 5' RACE was also performed to obtain the sequence of the 5' part of the mRNA but the attempt failed, again due to the low level of expression and also due to the large size of the missing portion of the mRNA. We do not know, therefore, the location of the promoter region of the gene. One can consider that the *U2mu* transcript is a splicing variant of the *Murr1* transcript that is formed due to the new splicing sites created by the transposition of the *U2af1-rs1* sequence into the intron of the *Murr1* gene, although it is also possible that the *U2mu* transcript is possible to be transcribed from a different promoter. We do not know which possibility is correct, but the former case seems more likely at present.

We do not know whether the *U2mu* transcript has any functions in mice. *U2mu* mRNA has an ORF that starts in the sequence from the *Murr1* intron, continues to the sequence from *U2af1-rs1* and is connected to the sequence from the *Murr1* exon that is shared by the *Murr1* ORF (Fig. 2). We have not identified the protein coded by the ORF yet, but the ORF may be functional because a long sequence (342 bp) is connected in-frame to the 3' half of the *Murr1* ORF without interruption by stop codons. The ORF in the *U2mu* transcript suggests that the transcript

AACTCACAGGATCCACTGCGTCTGTCTCCTAAGTGTGGGATTAATGGTCTGTAATACCA	60
GGTGGTTTGGGGGAGTCAAGTCCAGATGGTCAGGCTTGGCAGCAGTGTCCCTTCCTGCT	120
GAGCCATCTCATTAGCCCATCCATCTTTAATAAAAAGGGAACATACATAGATATATGATGT	180
AAGGGTGTCTAGTATTTACAAGCAGAAGAACTGTGGACAGAAGGCATTCTTTCTGCCA	240
GATTTTTTTTCCTTTAAACTAAAATCTACTCATTTTATTTTCACAGTTCATGATACAAAT	300
GAACTACTTTAAAAAAAAGTCTCTCTACTCCTAGCCAAAACCTTCCCTACGCTAGATA	360
TGGGGAGAAGTTTGCTTGTCATACCACTATAAAATACTCCGGTGTAGCCCCATGGTCTGA	420
AACAGAAAAGCAGGGAACTGATGATCCCCCTGTTCTTACCTGTCTGCCTGCCTGTGCCATG	480
CCATGCCAGGTGAGTGCCTCATCTCACTGGAGGCCTGGACACCAAGGGGATGTGTCTCT	540
CATGGGTGGCCTCATTTGGAGGAACAGGTCCCACAGGCATCACCCAGGAGGAGGGGCTGC	600
M G G L I G G T G P T G I T P G G G A A	
TACTGGCATCATGGAGGTGTCTCCCATGTTGGGTGCTTGCATCATACCAGAGTGTGGAGG	660
T G I M E V S P M L G A C I I P E C G G	
ACCCCGGGGACTGGAGCCAGAATTCACACTGGAGCTGTCTGCTGCGTACCATCTTCCAT	720
P R G L E P E F T L E L S S C V P S S I	
TAAAGAGAGAGGGGCTGCTTGGCATTCCTTCTCCGACTGGTACTGAACATACACATTGC	780
K E R E G C L A F F F R L V L N I H I A	
CCCCGAGATGAGGTTCCAGGTTGCAGCTTACTTTGAAGTGAATCACCTTTCCACGTTCT	840
P Q M R F Q V A A Y F E L N H L S H V L	
TGAAGTCCGGCAGCACGTCATGGTAGAAATCCAAGAACTGCTGTCTATTGCATCTGCAGA	900
E L R Q H V M V E I Q E L L S I A S A D	
CATGGATTTCACACAGTTAGAGGCATTCCTGACTGCTCAAACCAAAAAGCAAGGTGGCAT	960
M D F N Q L E A F L T A Q T K K Q G G I	
CACCAGTGAGCAAGCTGCAGTCATCTCCAAGTTTTGGAAGAGCCACAAGATAAAAATCCG	1020
T S E Q A A V I S K F W K S H K I K I R	
AGAGAGTCTCATGAAGCAGAGCCGCTGGGACAACGGCCTTCGGGGCCTGAGCTGGAGAGT	1080
E S L M K Q S R W D N G L R G L S W R V	
CGATGGCAAGTCTCAGTCACGGCACTCAACTCAGATACACAGCCCTGTTGCCATAATAGA	1140
D G K S Q S R H S T Q I H S P V A I I E	
GCTGGAATTTGGAAAAAATGGACAGGAATCTGAATTTTTGTGTCTGGAATTTGATGAAGT	1200
L E F G K N G Q E S E F L C L E F D E V	
TAAAGTCAAGCAATCCTGAAGAAGCTGTCTAGAGGTAGAAGAGAGTATCAACAGGCTGAT	1260
K V K Q I L K K L S E V E E S I N R L M	
GCAGGCAGCCTAACTGAAGAGAGTATCAATAGGCTGATGCAGGCAGCCTAACTGAAGGCT	1320
Q A A *	
GGAGGAAGGGGCGTTTGAAGTGAAGCTGCTCACAGACTTTCTCCACTGACCCTTTGAAAG	1380
TCCTGTTTGGCCACTGGTGTACCAAAAGACATTGTATACATGCATGAAAGTCTTCAAGA	1440
ATAAAATAAAATATATTTT-poly-A	1459

Fig. 2. Nucleotide sequence of *U2mu* cDNA and the deduced amino acid sequence. The shaded sequence from #1 to #679 comes from the intron of the *Murr1* gene. The underlined one from #680 to #883 is an antisense sequence of *U2af1-rs1* and the last part is from the last exon of *Murr1* (see Fig. 1). The 3' portion of the ORF is identical to that of the *Murr1* ORF (data not shown). The boxed sequence (#1029–1034) is a polymorphic *Bsp*HI site used for the analysis of allelic expression of the *U2mu* gene. The polyA signal is underlined. The two arrows indicate the positions of the primers, UMUF and UMUR2, used for the analysis of allelic expression in Fig. 4.

has a function different from that of the *Murr1* transcript.

In the evolution of eukaryotes new genes are thought to emerge mainly through two processes, that is, gene duplication followed by divergence of the duplicated genes and shuffling of exons derived from different genes by unequal crossing-over or transposition (Ohta, 2000). The

U2mu transcript, however, does not result from either of these processes. This transcript consists of sequences from at least three exons. The second exon is transcribed from a sequence that is thought to have transposed from another location where the sequence was not transcribed in this direction. We are interested in the

Fig. 3. Northern blot analyses of *U2mu* and *Murr1* in adult mouse tissues. a) Probed with *U2mu* probe, exposed for 2 weeks with an intensifying screen. b) Probed with *Murr1* probe, exposed for 24 hours with an intensifying screen.

Fig. 4. Biallelic expression of *U2mu* transcript. RT-PCR was performed on polyA RNAs from the tissues of adult mice. The 531 bp cDNA products were digested with *Bsp*HI. B6 cDNA was cut with the enzyme (lane B6), whereas PWK cDNA was not (lane PWK). Both alleles were expressed in all the tissues examined of F1 hybrid mice between these strains, BPF1 (B6 female x PWK male) and PBF1 (PWK female x B6 male).

process of the formation of the *U2mu* transcript (whereby the transposed sequence was connected as a new exon to preexisting sequences, the intron and an exon sequence of the *Murr1* gene) because this may be a new example of the process of the emergence of a new gene. It may be useful to identify which animals have homologues of *U2af1-rs1* and *U2mu*, and which animals do not in the phylogenetic tree. Comparison of the corresponding sequences among these animals may give important insights into the process of the emergence of the *U2mu* transcript.

We thank Mr. S. Takuma at the Center for Laboratory Animals for his technical assistance. This work was supported by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology in Japan.

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