



Identification of a Chimeric Transcript formed by Intergenic Splicing of *UbiE2* and *Yghl1-4* in Mouse

Ravi Nagganatha Suganthan, Sahi Vaidurya Pratap, Morita Eugene Hayato and Abe Shunnosuke*

Laboratory of Molecular Physiology, Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama 790-8566, Ehime, JAPAN

Available online at: www.isca.in, www.isca.me

Received 26th October 2013, revised 6th December 2013, accepted 4th January 2014

Abstract

We identified a chimeric transcript *UbiE2-Yghl1-4*, formed by an intergenic splicing event between *UbiE2* and *Yghl1-4* present in tandem position on the mouse chromosome 15F1 at a distance of ~3kb. The *UbiE2* is S-adenosylmethionine-dependent methyltransferase domain containing protein encoding gene, also known as *mettl7a2*. The *Yghl1-4* is a member of *YGHL/HIG1* gene family in mouse with *YGHL/HIG1* exon 3 domain. The chimeric transcript is formed with exon 1 of *UbiE2* fused to exons 2-4 of *Yghl1-4* using the canonical splice sites from both genes. The *UbiE2-Yghl1-4* is not a product of in vitro artifacts and our results showed independent expression of the parent and the chimeric transcripts. The expression of *UbiE2*, *Yghl1-4* and *UbiE2-Yghl1-4* were found exclusive to kidney only. Further, tissue localization by Non-Radioactive In Situ Hybridization (NRISH) indicated restricted expression in the epithelium of proximal tubules and adrenal cortex. The *UbiE2-Yghl1-4* gene formation was found only in mouse and not in human. The characteristics of the *UbiE2-Yghl1-4* chimeric transcript being specific to mouse genome and containing both the methyl transferase domain and the *YGHL/HIG1* exon 3 domain appears to have functional implications in mouse and especially in the kidney, rather than merely generating genetic diversity.

Keywords: Chimeric transcript, *UbiE2*, *Yghl1-4*, *YGHL/HIG1*, mouse, kidney.

Introduction

RNA processing, other than conventional splicing and polyadenylation events, includes random combination of exons producing different variants of mRNAs. The alternative splicing process is not just restricted to the single gene but include exons from other genes also^{1,2}. The phenomena of both alternative cleavage and splicing between two different or tandem genes is known as intergenic splicing which produces chimeric transcripts, thus increasing transcript and protein diversity³⁻⁵. Two major mechanisms leading to chimeric transcript formation are (a) trans-splicing between transcripts of unrelated genes and (b) formation of read-through transcripts between neighbour genes⁶⁻⁸. These chimeric transcripts have also been shown to express as an entity independent of the parent transcripts⁹. Chimeric transcripts were considered to be a rare phenomenon a decade ago, but till date numerous chimeric transcripts have been identified and reported^{3-5, 10-18}.

The yellowtail growth hormone like-1 (*YGHL1*) cDNA (GenBank: D85880) was obtained on screening a pituitary cDNA library of *Seriola quinqueradiata* (Yellowtail fish or Japanese Amberjack) using the antibody for Salmon growth hormone by our research group in 1996¹⁹. Later, Gracey *et al.*²⁰ identified the Hypoxia-Inducible Gene 1 (*HIG1*) cDNA (GenBank: AF266206), in liver tissue of a euryoxic fish, *Gillichthys mirabilis*, shown to be induced after prolonged hypoxia. The *HIG1* and the yellowtail *YGHL1* were found to be homologous, thus belonging to the *YGHL1/HIG1* gene family.

Multiple paralogs of the *YGHL1/HIG1* are cloned and characterised in various eukaryotic genomes²¹⁻²⁴. The generation of multiple copies of these mammalian gene families has been attributed to ancient polyploidization in ancestral vertebrates²⁵⁻²⁹. There are four paralogs of *YGHL1/HIG1* in mouse *viz.* *Higd1a* (9F4), *Higd1b* (11D), *yghl1-4* (15F1) and *Higd2a* (13B1)¹⁹.

We describe a chimeric transcript as a result of intergenic splicing in mouse involving the *UbiE2* (*Mettl7a2*) and *Yghl1-4* (*Higd1c*). These two genes are situated ~3 kb apart from each other on mouse chromosome 15F1 in tandem position. The *UbiE2* is a S-adenosylmethionine-dependent methyltransferase domain containing protein encoding gene also known as *mettl7a2* with unknown function existing in vertebrates while the *Yghl1-4* is a member of *YGHL1/HIG1* gene family in mouse with *YGHL1/HIG1* exon 3 domain¹⁹. The intergenic splicing product of *Yghl1-4* with its flanking gene *UbiE2* was found as a fusion gene through EST database searches. The chimeric gene is composed of the exon 1 from *UbiE2* and the exons 2-4 from the *Yghl1-4*, with a continuous open reading frame from the start codon in *UbiE2* and the stop codon in *Yghl1-4*.

This chimeric transcript must putatively have a novel function in mouse as it does not exist in human. We describe the intergenic splicing event of the *UbiE2* and *Yghl1-4* and also the expression patterns of the individual and chimeric transcripts in the mouse.

Material and Methods

Isolation of total RNA, determination of cDNA extremities, and cDNA analysis: RNA was extracted from various organs of mouse using an RNA Extraction Kit (GE Healthcare, USA). The 5'cDNA extremity was determined by the rapid amplification of cDNA ends (RACE) method. Poly (A⁺) RNA was purified from total RNA (100 µg) using an mRNA Purification kit (GE Healthcare, USA). After annealing 1 µg of poly (A⁺) RNA with 10 pmoles of a gene specific reverse primer (5'-gtggagataggcacagtgtgat-3') derived from the partial cDNA sequence, the first strand cDNA was synthesized by incubation with ReverTraAce reverse transcriptase (Toyobo, Japan) for 1 h at 42 °C. Then, the blunt ended second strand was synthesized by simultaneous inclusion of DNA polymerase I (9U/µl), *E. coli* DNA ligase (6U/µl, Takara, Japan), and T4 DNA polymerase (9U/µl) in the presence of RNase H (1U/µl) in a reaction buffer composed of 33.4 mM Tris-HCl (pH 8.5), 4.6 mM MgCl₂, 10 mM (NH₄)₂SO₄, 6.6 mM 2-mercaptoethanol, 0.06 mM EDTA, 0.005 % BSA, 100 mM KCl, and 0.15 mM NAD at 16 °C for 90 min., and the double-stranded cDNA obtained (5 µl) was ligated with 12.5 pmoles of an adaptor consisting of complimentary oligo DNA, (5'-gtaatacagactactatagggcacgcgtggtcgacggccgggctgtgt-3') and (5'-accagcccggg-3'), using 4 U/µl T4 DNA ligase at 16 °C overnight. T4 DNA ligase, DNA polymerase I, and RNase H were obtained from Toyobo, Japan. The ligated cDNA fragments were 5'-RACE amplified by using an adapter primer (5'-gtaatacagactactatagggc-3') with gene specific primers in 25 µl volume reaction mixtures using XL-PCR kit (Applied Biosystems, USA), and then subcloned into the pT7blue vector.

Analysis of gene expression by RT-PCR: For RT-PCR analysis, the cDNA from various mouse tissues were diluted to optimize the analysis of *UbiE2*, *Yghl1-4*, *UbiE2-Yghl1-4* and β -*actin*. The primer sequences are F-5'-ATGCCCTTTCTCCTCTACTCGC-3' and R-5'-AACCAGCGTGGAGCAGACCGG-3' for *UbiE2* amplification; F-5'-ACTGGGATACTGTTCCAGC-3' and R-5'-TCTGATGCTAGCACTG-3' for *Yghl1-4* amplification; and F-5'-ATGCCCTTTCTCCTCTACTCGC-3' and R-5'-TCTGATGCTAGCACTG-3' for the chimeric transcript amplification. The XL-PCR kit (Applied Biosystems) was used for carrying out all RT-PCR reactions. The reaction consists of an initial denaturation for 15 s at 94 °C, then 30 cycles of: 15s at 94 °C, 1 min at 60 °C, 2min at 72 °C, and then a final extension for 10 min at 72 °C. The PCR products were electrophoresed in 0.5% TAE composed of 20 mM Tris (pH 8.0), 9.5 mM acetic acid and 0.5 mM Na₂EDTA; the gel irradiated at 310 nm and photographed using a gel documentation system (Pharmacia Biotech).

DNA sequencing and analysis: Subcloned DNA was isolated and purified using a FlexiPrep kit (GE Healthcare, USA), and sequenced using a Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA) and an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, USA).

Non-radioactive mRNA in situ hybridization: NRISH using biotin-labeled antisense riboprobes for *UbiE2*, *Yghl1-4* and the chimeric transcript was performed on parasagittal sections of mouse embryo (Genostaff, Japan). The gene specific biotin-labeled riboprobes were synthesized from the sequences cloned in pGEM-T vector (Promega, USA) by *in vitro* transcription using RNA biotin labeling mix (Roche, Germany) and Thermo T7 polymerase (Toyobo, Japan). Sections were deparaffinized and rehydrated in serial dilution of ethanol, then incubated overnight at 42 °C with the riboprobes in hybridization buffer. Thereafter, the sections were sequentially washed with 4 \times , 2 \times , and 0.2 \times SSC buffer at 42 °C for 1 h each. Detection was achieved by treatment with 1 \times PBST (Phosphate buffered Saline + 0.1 % Tween 20) for 5 min, followed by treatment with blocking buffer for 3 h. The sections were treated with the alkaline phosphatase-streptavidin conjugate overnight at 4 °C. Subsequently, the sections were washed 3 times with 1 \times PBST for 15 min and then washed with alkaline Tris buffer. To detect the hybridized probe, the sections were incubated with nitro blue tetrazolium (NBT; 450 mg/ml) and 5-bromo-4-chloro-3-indoyl phosphate (BCIP; 175 mg/ml) in alkaline Tris buffer at 37 °C. As internal controls, NRISH using sense riboprobes were performed.

Results and Discussion

Identification and expression analysis of *UbiE2-Yghl1-4* intergenic splicing product: An EST sequence (Genbank: CA481201) containing sequences of *UbiE2* and *Yghl1-4* was found while searching the EST database for sequences related to *YGHL/HIG1* in mouse genome. The NCBI blast search with the EST sequence showed high similarity with exon 1 of the *UbiE2*; with exons 2, 3 and partially with exon 4 of *Yghl1-4*. To validate the EST based search, RT-PCR was performed on cDNAs from various mouse organs, using gene specific primers designed for the *UbiE2* and *Yghl1-4* and the *UbiE2-Yghl1-4* chimeric transcript (figure 1). Relative expression of the different transcripts was normalized using the β -*actin* as control. Interestingly, both the parent transcripts and the chimeric transcript were amplified in a tissue specific manner, expressing abundantly in the kidney only. The sequencing of the chimeric transcript PCR product showed that it is an intergenic splicing product containing the regions of *UbiE2* and *Yghl1-4*.

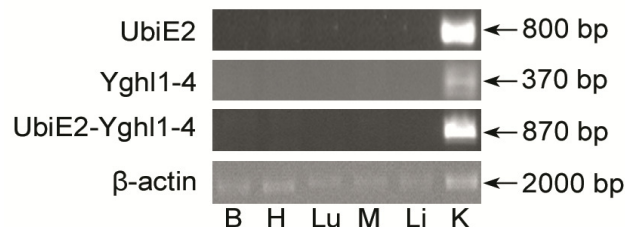


Figure-1
RT-PCR amplification of the *UbiE2* and *Yghl1-4* and *UbiE2-Yghl1-4* chimeric transcript and β -*actin* in mouse tissues (B - Brain, H - Heart, Lu - Lung, M - Muscle, Li - Liver, K - Kidney)

Genomic organization of *UbiE2* and *Yghl1-4* loci and structural analysis of *UbiE2-Yghl1-4*: The genomic sequence analysis showed that the *UbiE2* and the *Yghl1-4* are present in the mouse chromosome 15F1. *UbiE2* contains two exons and the *Yghl1-4* contains four exons. The *Yghl1-4* is situated 2766 bp upstream of the *UbiE2* and both genes are in tandem, with the same orientation. The Intergenic splicing product encompasses *UbiE2* exon 1, fused to *Yghl1-4* exons 2–4 using the splice donor/acceptor sites of *UbiE2* and *Yghl1-4* mRNAs, along with the 3' UTR and the polyadenylation site of *Yghl1-4* (figure 2). The intergenic splicing event removed the exon 2 of the *UbiE2* and exon 1 of the *Yghl1-4* as a novel intron region. The genomic organization of the loci, together with the structure of the intergenic splicing product, were compatible with intergenic splicing between *UbiE2* and *Yghl1-4*, giving rise to the *UbiE2-Yghl1-4* transcript. The complete nucleotide sequences of the *UbiE2* (Genbank: AB253944), *Yghl1-4* (Genbank: AB253942) and the chimeric transcript (Genbank: AB110950) were deposited in NCBI database (figure 3).

Localization of *UbiE2*, *Yghl1-4*, and *UbiE2-Yghl1-4* chimeric transcript in mouse: To confirm the tissue-specific expression of *UbiE2*, *Yghl1-4* and *UbiE2-Yghl1-4* chimeric transcript in the mouse, NRISH was carried out on parasagittal sections of the mouse embryo using gene specific antisense riboprobes. The *UbiE2* and *Yghl1-4* hybridization signal were strongly detected in the kidney, whereas the signal was weak for the chimeric transcript (figure 4A). The magnified observation showed similar levels of expression but restricted to the epithelium of the proximal tubules (figure 4B) whereas, strong expression for all the transcripts in the adrenal cortex region (figure 4C) was observed.

Discussion: Mouse EST database search for sequences related to *YGHL/HIG1* revealed the presence of a chimeric *UbiE2-Yghl1-4* transcript. This chimeric transcript seems to be an intergenic splicing product between *UbiE2* and *Yghl1-4*. Sequence analysis indicated that *UbiE2-Yghl1-4* chimeric transcript encompass *UbiE2* exons 1, including the AUG initiation codon, fused in-frame to *Yghl1-4* exons 2–4. There is no evidence of such a mechanism affecting these two genes in human. *UbiE2* and *Yghl1-4* genes map to mouse chromosome 15F1, are located in tandem and are separated by ~3 kb.

Unconventionally, chimeric transcripts are formed from experimental artefacts due to template-switching activities of the reverse transcriptase (RT) or *Taq* polymerase³⁰. The formation of *UbiE2-Yghl1-4* has followed splicing using canonical splice sites whereas template switching activity is independent of splice sites. There is no sequence similarity between *UbiE2* and *Yghl1-4* that could contribute to a homology-dependent template switching. Moreover, the chimeric transcript was identified based on an EST that was obtained in the mouse EST database. The chimeric transcript consists of both the S-adenosylmethionine-dependent methyltransferase domain from the *UbiE2* and the *YGHL/HIG1* exon 3 domain from the *Yghl1-4*, so it appears to have functional implications, rather than merely generating genetic diversity, as previously hypothesized for other hybrid RNAs¹⁰. These characteristics showed that *UbiE2-Yghl1-4* is not a product of *in vitro* artefact, but that its expression is tightly regulated, and has a functional significance.

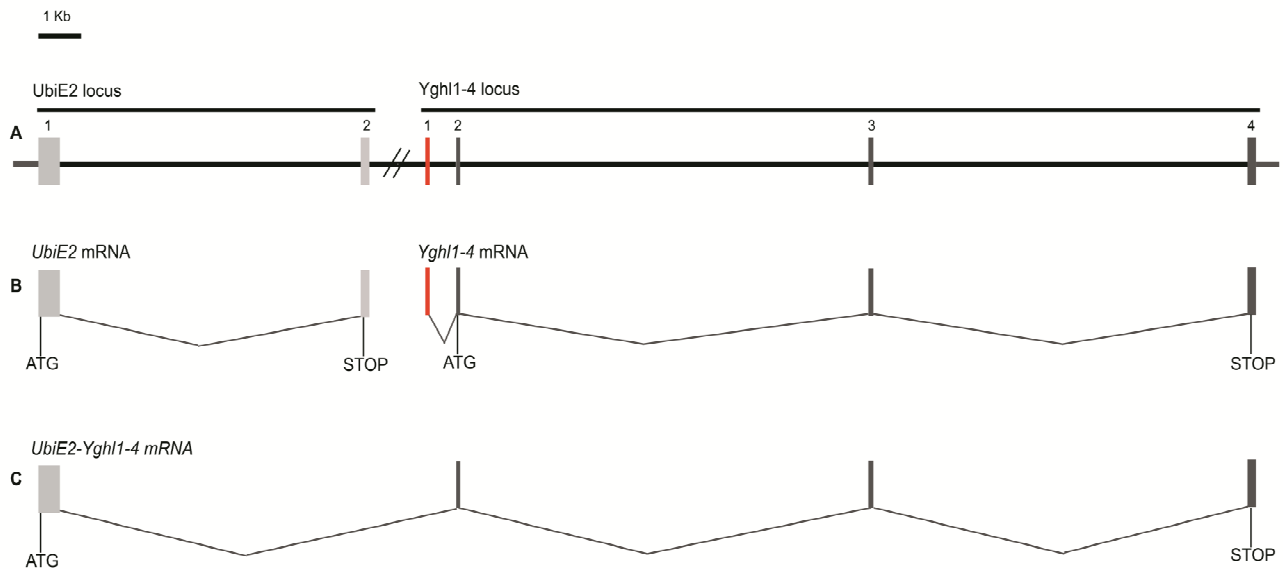


Figure-2

Structure of the mouse *UbiE2* and *Yghl1-4* genes and transcripts, A- Genomic structure of the two genes. B- Structure of the normal transcripts from both genes. C- Structure of the chimeric transcript *UbiE2-Yghl1-4*. Untranslated exon is in red and translation start and stop are indicated

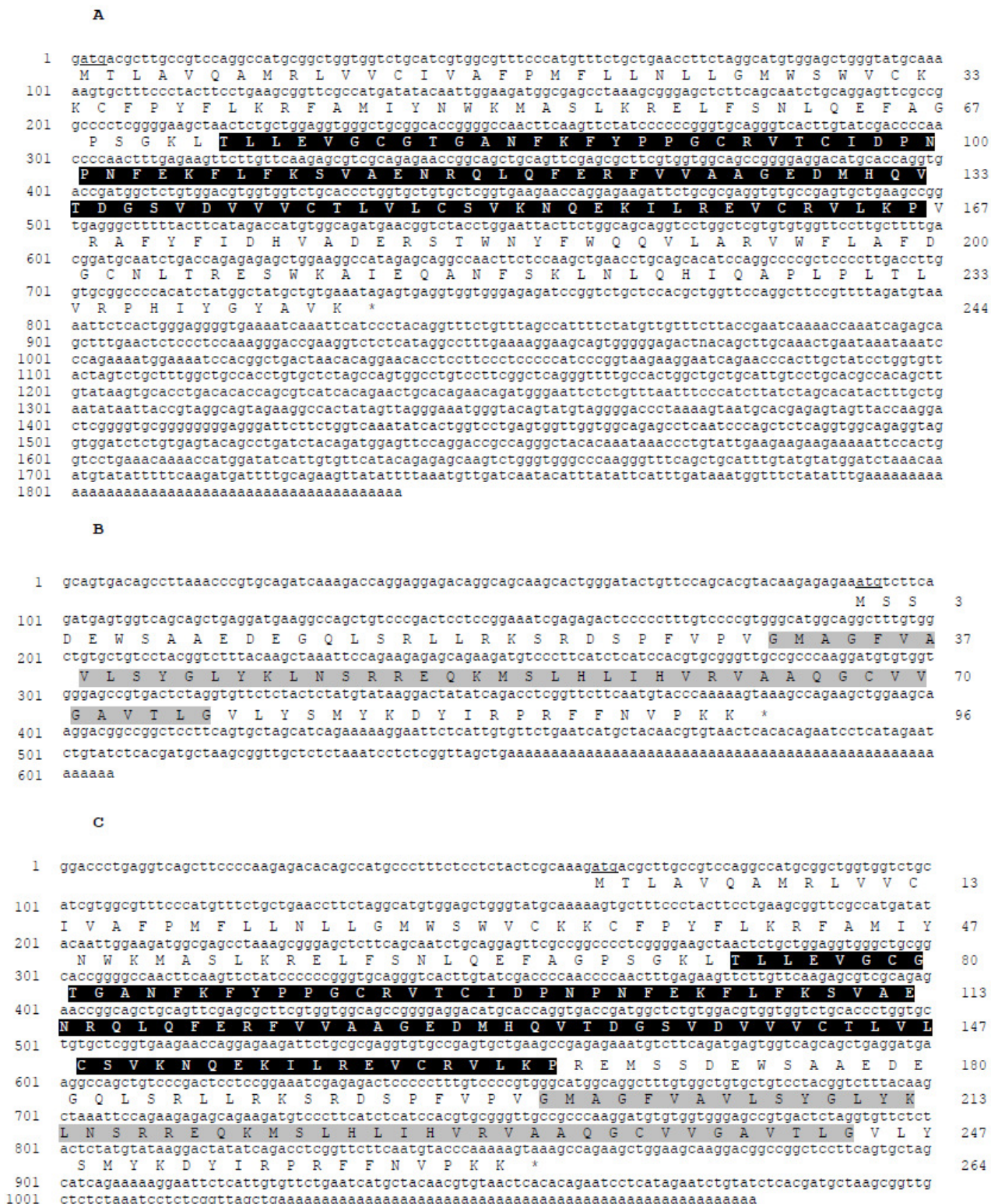


Figure-3

Nucleotide sequence and deduced amino acid sequence of *UbiE2* (A), *Yghl1-4* (B) and *UbiE2-Yghl1-4* chimeric mRNA (C). The initiation codon (atg) is underlined and the stop codon (taa/tag) is indicated by an asterisk. The S-adenosylmethionine-dependent methyltransferase domain and the *YGHL1/HIG1* exon 3 domain sequences are highlighted in black and grey respectively

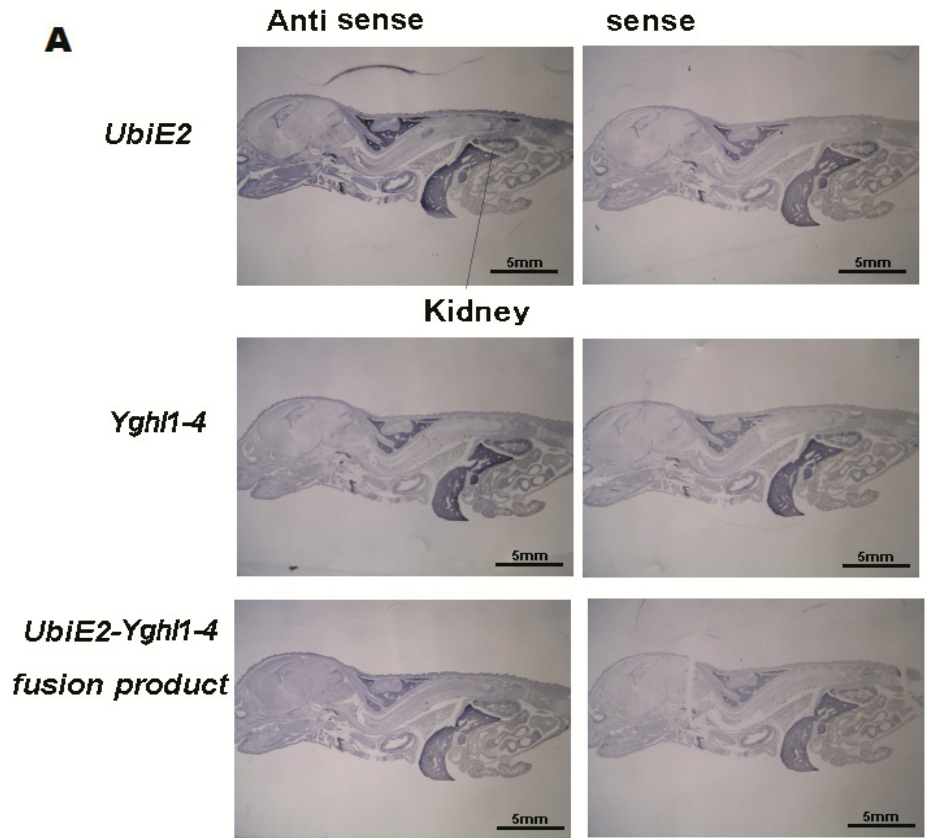


Figure-4(A)

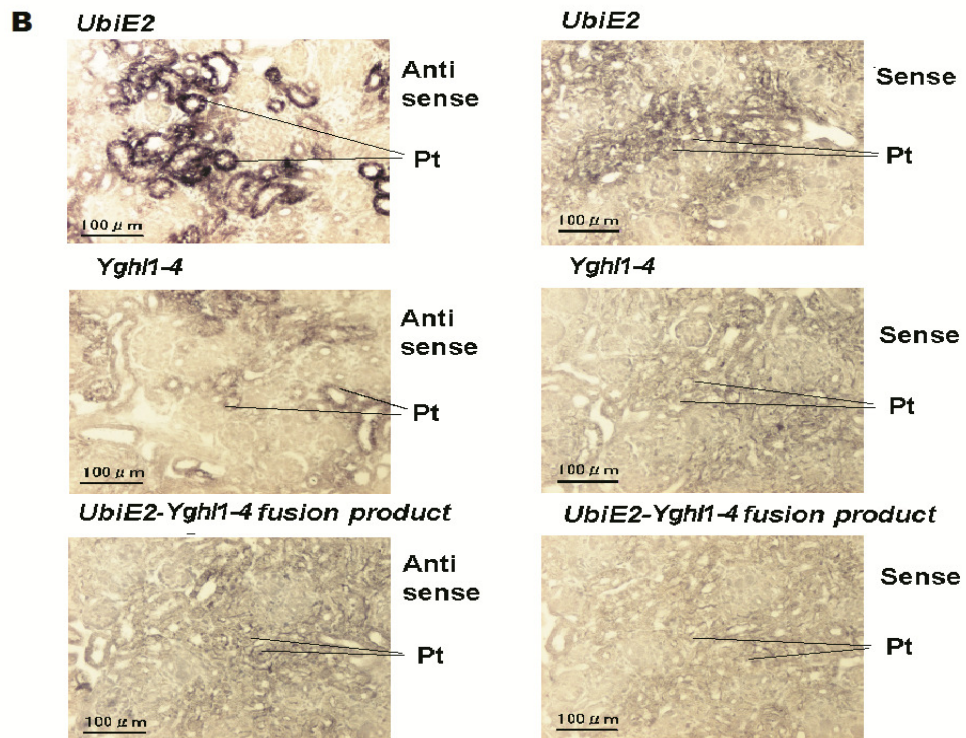


Figure-4(B)

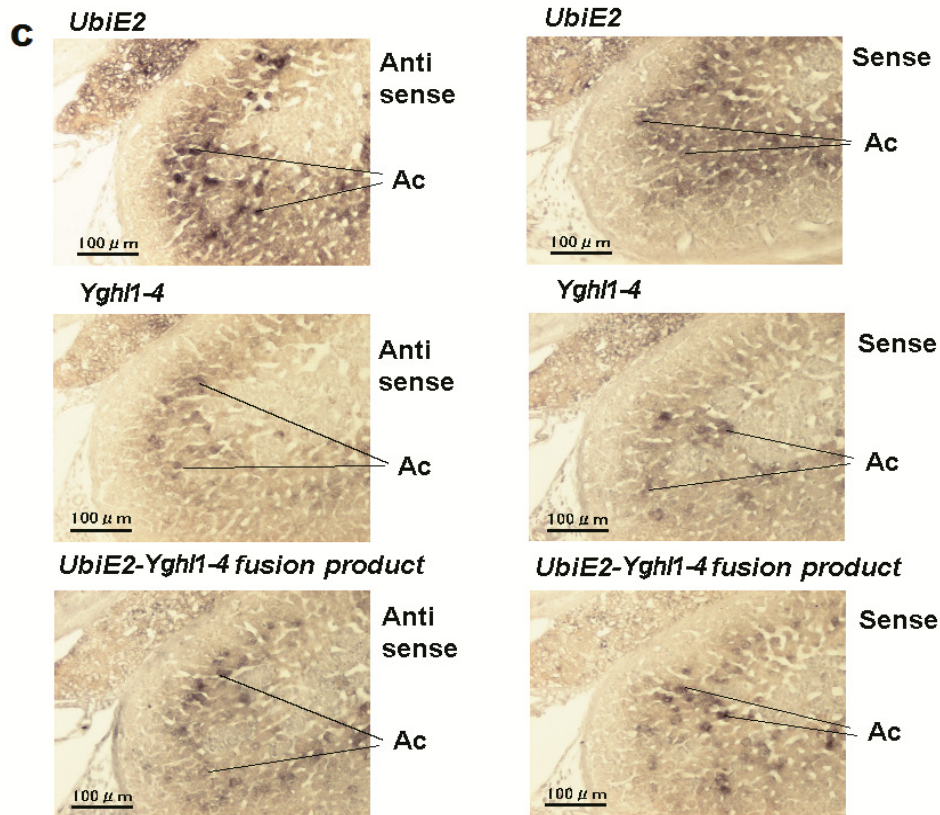


Figure-4

Expression of *UbiE2*, *Yghl1-4* and *UbiE2-Yghl1-4* chimeric mRNA in parasagittal sections of mouse embryo hybridized with antisense or sense biotin-labeled riboprobes. The signal detection shows *UbiE2*, *Yghl1-4* and *UbiE2-Yghl1-4* chimeric mRNA expression in the kidney (A), proximal tubules (Pt) (B) and the adrenal cortex (Ac) (C)

Several mechanisms by which the chimeric transcripts generate have been reported previously, such as trans-splicing^{2,31,32}, RNA transcriptional runoff^{4,17}, or by presence of short homologous sequences³³. The *UbiE2-Yghl1-4* chimeric transcript formation has followed the classical intragenic trans-splicing model using the canonical splice sites (GT-AG) which was also consistent in reported intergenic trans-splicing events^{11, 34-36}. The intergenic splicing event of the *UbiE2-Yghl1-4* has occurred between the exon 2 of *UbiE2* and exon 2 of the *Yghl1-4*, thus creating a novel intron, spanning the region between the 5' splice site (donor) of the last intron and the 3' splice site (acceptor) of the first intron of the *UbiE2* and *Yghl1-4*, respectively. Akiva *et al.*⁴ reported that the former intergenic splicing type is the most abundant type in human chimeric transcript formation and classified it as “exon (n-1) to (+2) event”. The exon (n-1) to (+2) event occurs as an intergenic splicing between the n-1 exon (one before last) of the upstream gene and the second exon (+2) of the downstream gene. It should be also noted that the *UbiE2-Yghl1-4* chimeric transcript was not a result of a weak termination signal for *UbiE2* transcription; because RT-PCR analysis showed that normal *UbiE2* and *Yghl1-4* transcripts were also expressed, meaning that normal transcription termination and intergenic splicing of the two genes co-exist.

Even though, the *UbiE2* and *Yghl1-4* orthologs are conserved on human chromosome 12q13.12 as *METTL7A* and *HIGD1C*, with a distance of ~21.5 kb between them, there is no evidence in the human cDNA or EST databases implying the chimeric transcript. Roux *et al.*⁵ pointed out that for several cases of chimeric transcript formations described so far, the physical distance between the genes involved does not seem to have any influence. The genes P2RY11 and PPAN are separated by only 400 bp¹³, whereas the DISC1 and TRAX genes are separated by 35 kb³⁷. Although the exact reason for the absence of the chimeric transcript in human is not known, the structure and sequence of the human *HIGD1C* which is different from the mouse *Yghl1-4*, may explain the fact to certain level. The *HIGD1C* lacks the non-coding exon 1 as in *Yghl1-4*, which may be some sort of signal for the intergenic splicing to occur. The absence of the non-coding exon 1 in human counterpart may pose a hindrance to follow the mechanism as occurring in mouse. Secondly, the canonical AG acceptor site is absent at the 5' end of coding exon 1 of the *HIGD1C* for the reading frame to join with the exon 1 of the human *METTL7A*. These may be the reasons that the reading frames of both genes in human cannot be joined as in the mouse chimeric transcript. The occurrence of the intergenic splicing event between *UbiE2* and *Yghl1-4* only in mouse may be due to some specific genomic requirements

and the resultant chimeric transcript may have well defined functional role. Prakash *et al.*³⁸ reported the presence of 270 conjoined genes in mouse genome and also that only 0.03% of the mouse conjoined genes junction exons are found conserved in human, which indicates that many chimeric transcripts might have undergone evolutionary selective pressure to be present in the mouse genome only. Naganuma *et al.*¹⁴ reported a chimeric mRNA transcribed from *HAI-2* and *H2RSP* in human but not to be found in mouse; and Roux *et al.*⁵ reported a bovine chimeric transcript between *PPARG* and *TSEN2* specific to cattle but absent in human and mouse genomes.

Frenkel-Morgenstern *et al.*⁸ reported that chimeric transcripts, irrespective of expression levels, are significantly more tissue specific than non-chimeric transcripts. The chimeric transcript we report here, showed a tissue expression pattern in the kidney. Also the parent transcripts showed tissue specific expression in the kidney which was also confirmed by the NRISH data. The NRISH data also showed selective expression of the parent and chimeric transcripts in epithelium of the proximal tubules and adrenal cortex. So we speculate that the *UbiE2*, *Yghl1-4* and the *UbiE2-Yghl1-4* may exert some important physiological role in the kidney which has to be confirmed with more empirical data.

Conclusion

We have described a chimeric transcript *UbiE2-Yghl1-4* as a result of intergenic splicing event between *UbiE2* and *Yghl1-4*. The existence of the *UbiE2-Yghl1-4* appears to be restricted to mouse and its expression is further restricted to kidney tissues. The physiological and functional role of the parents and the chimeric transcript are to be elucidated in future studies.

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