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RESEARCH PAPER

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The alternative splicing of *SKU5-Similar3* in *Arabidopsis*

Ke Zhou

FAFU-UCR Joint Center for Horticultural Biology and Metabolomics, Haixia Institute of Science and Technology, Fujian Agriculture and Forestry University, Fuzhou, China

ABSTRACT

Alternative splicing largely enhanced the diversity of transcriptome and proteome in eukaryas. Along with technological development, more and more genes are reported to be alternatively spliced during mRNA maturation. Here, I report the alternative splicing of *SKU5-Similar 3* (*SKS3*) and its special splicing site in *Arabidopsis*. *SKS3* was predicted to be alternatively transcribed into two variants, *SKS3.1* and *SKS3.2*, which encoded a GPI-anchored protein and a soluble secretory protein, respectively. But, according to experimental data, instead of *SKS3.2*, a novel variant, *SKS3.3*, which encodes a protein with a transmembrane region at its C-terminus, was demonstrated. Interestingly, it exhibites a different organ-specific expression pattern with *SKS3.1*, and an unusual intron splicing site not following 'GT-AG' rule or any reported rule.

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Introduction

In eukaryas, precursor messenger RNA (pre-mRNA) is transcribed directly from genomic DNA, and then introns are removed, and exons are spliced to be mature mRNA for translation.[1–3] The splicing of pre-mRNA is not always unique, but could be alternatively, which makes one gene transcribes diverse variants, and encodes various proteins.[4] It largely enhanced the diversity of transcriptome and proteome.[5,6] Along with the technological development, >95–100% of human genes,[3] and up to 60% of intron-containing genes of *Arabidopsis* genes,[1,2,5,7] are found alternatively spliced and produce at least two alternative variants. The alternative splicing processes are catalyzed by the protein-RNA complexes, spliceosomes, which could recognize the 5', 3' splice sites and the branch point of introns.[8–10] This recognition and splicing mechanism results in "GT-AG" rule that, most introns start from "GT" and end at "AG".[4,8]

Interestingly, the alternative splicing is alternatively that, its occurrence and expression pattern could be regulated by various abiotic stresses,[11–15] developmental stages,[16] or different organs.[17] Although the exact mechanism hasn't been revealed thoroughly yet,[18] a few reports indicated its importance during development, such as, the different alternative variants of *HAB1* play opposite roles during ABA signaling in *Arabidopsis*.[19]

SKU5-Similar 3 (*SKS3*) belongs to a subgroup of *SKU5-Similar* gene family that encode GPI-anchored proteins and are redundantly essential for cell polar expansion and cell wall synthesis of roots in *Arabidopsis*. *SKS3* is predicted to alternatively encode asoluble secretory protein and aplasma membrane attached GPI-anchored protein.[20] In this study, I report an unexpected organ-specific alternatively spliced variant of *SKS3* in *Arabidopsis*, which could encode a plasma membrane attached protein with transmembrane region at C-terminus, besides the predicted variant encoding a GPI-anchored protein. Interestingly, its splicing site seems unique, which does not follow the "GT-AG" rule, or any other reported rules. But due to the short repeated "ATCCATC" localizing close to the borders of spliced intron, the exact site could not be identified.

Results

Predicted alternative splicing of SKS3 gene

According to NCBI (https://www.ncbi.nlm.nih.gov/) and TAIR (http://www.arabidopsis.org/) database, two transcriptional variants, *SKS3.1 and SKS3.2* are predicated to be transcribed from *SKS3* gene. *SKS3.1* encodes aGPI-anchoredprotein precursor containing 589 amino acid residues, which could be modified with a glycosylphosphatidylinositol modification at C-terminus; and *SKS3.2* encodes asecretoryprotein precursor containing 614 amino acid residues. Gene structures of *SKS3.1* and *SKS3.2* show that, the predicted alternative splicing occurs at the last exon where the STOP codon of *SKS3.1* is removed together with the last intron of *SKS3.2* (Figure1).

Observed alternative splicing of SKS3 gene in vivo

In *Arabidopsis*, *SKS3* variants were cloned from cDNA generated from different organs. Surprisingly, instead of *SKS3.2*, a novel variant, *SKS3.3*, was identified, and exhibit a different expression pattern with *SKS3.1* (Figure2(a)). *SKS3.3* encodes a shorter precursor containing 314 amino acid residues predicted to attach to plasma membrane through its C-terminal

CONTACT Ke Zhou zhouke0525@gmail.com FAFU-UCR Joint Center for Horticultural Biology and Metabolomics, Haixia Institute of Science and Technology, Fujian Agriculture and Forestry University, Fuzhou 350002, China

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Figure 1. Gene structures of *SKS3.1* and *SKS3.2* predicted by NCBI and TAIR database. Arrows showed the translation direction; exons are in dark grey; untraslated region (UTR) is in light grey; and introns are shown as black lines.

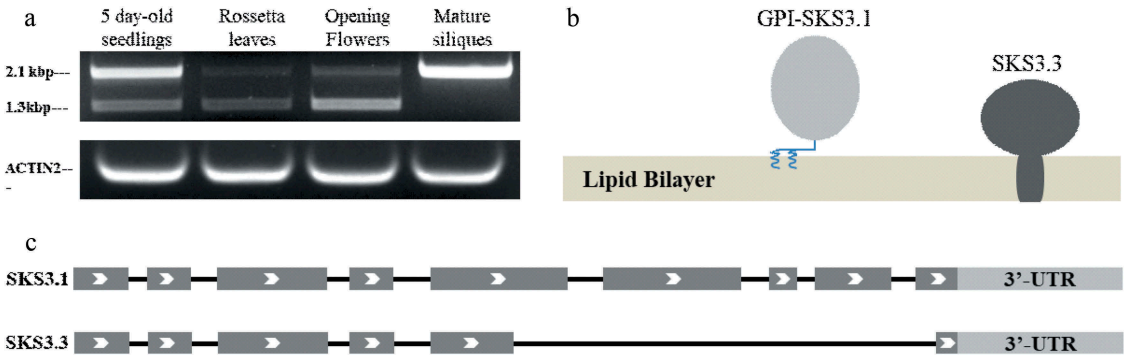

Figure 2. Alternatively splicing of *SKS3* gene in *Arabidopsis*. (a), two *SKS3* variants were amplified from cDNA extracted from different organs of *Arabidopsis*; Actin was utilized as reference and primers were indicated in methods; (b), predicted GPI-anchored SKS3.1 and transmembrane SKS3.3; (c). gene structure of *SKS3.1* and *SKS3.3*. Arrows show the translation direction; exons are in dark grey; untranslated region (UTR) is in light grey, and introns are shown as black lines.

transmembrane region (Figure2(b)). Gene structure of *SKS3.3* exhibits an unusual alternative splicing with a long intron cleavage including the whole 6th, 7th and 8th exons, partly 5th and 9th exons, and all introns between them(Figure2(c)).

The novel splicing site within "ATCCATC" of SKS3.3

Interestingly, instead of "GT-AG" rule, the borders of the spliced intron do not follow any reported splicing rule, but an unusual "ATCCATC" repeat was found close to the splicing site (Figure 3). However, due to the presence of this repeat, the exact splicing site of *SKS3. 3* could not be recognized yet, but limited within the short repeat "ATCCATC".

Discussion

Alternative splicing largely enhances the diversity of transcriptome and proteome, which allows one gene to encode various proteins. According to NCBI and TAIR databases, *SKS3* gene produces two transcriptional variants, *SKS3.1* and *SKS3.2*, which encode a GPI-anchored protein and a secretory protein respectively. But instead of *SKS3.2*, experimental data reveals another transcriptional variant, *SKS3.3*, which encodes a C-terminal transmembrane protein and exhibites different expression pattern with *SKS3.1* in various organs. It indicates the high complexity of alternative splicing in *Arabidopsis*.

Both GPI-anchored SKS3.1 and the smaller C-terminal transmembrane SKS3.3 are expected to attach to the external surface of plasma membrane. Differently, GPI anchoring is much more than a linkage to attach protein on plasma membrane, but plays essential roles in protein sorting and signaling transduction. It suggests a potential functional diversity of SKS3.1 and SKS3.3 protein.

Interestingly, *AT5G01745* encoding along non-coding RNA (lncRNA) was found reveresly at 3'-terminus of *SKS3*, overlapping with the alternative splicing site of *SKS3.3* variant (Figure4). LncRNA has been reported to be involved in alternative splicing, potentially through forming double-strand to prevent the recognition and splicing from spliceosome.[21–25] It suggests the potential involvement of lncRNA in the unusual alternative splicing of *SKS3*, and it would be very interesting to further investigate the connection between the alternative splicing and the lncRNA, and the regulation of this lncRNA.

Generally, due to the specific recognition and splicing by spliceosomes, the vast majority of introns start from "GT" and end at "AG",[4,8–10] with a few exceptions, such as starting from "GC" and ending at "
AG".[5] Interestingly, unusual intron borders were identified in *SKS3.3*variant. But due to the presence of the repeated "ATCCATC" close to it, the splicing site of *SKS3.3* could only be limited within these short repeats. It would be very interesting to investigate the exact splicng site and its potential regulation by the lncRNA.

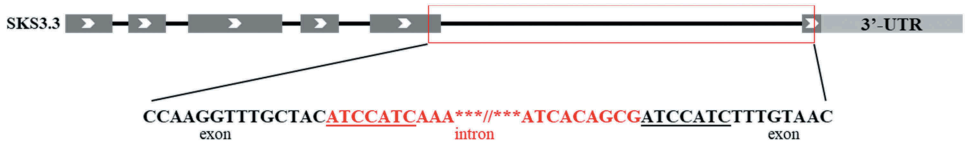

Figure 3. Splicing site of *SKS3.3* variant. Arrows showed the translation direction; exons are in dark grey; untranslated region (UTR) is in light grey, and introns are shown as black lines, the alternatively spliced intron is in red blank and the sequence close to splicing site is labeled in red, and the "ATCCATC" repeats are underlined.

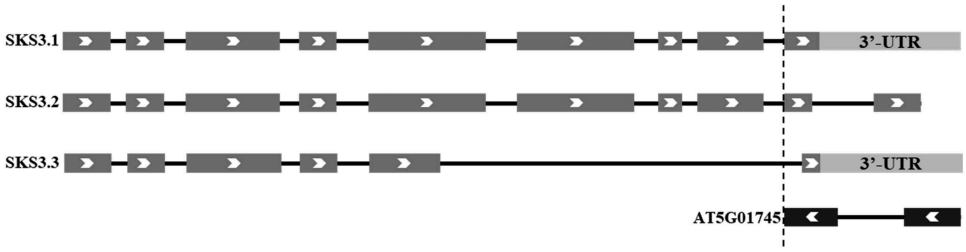

Figure 4. Transcriptional variants of *SKS3*, and the lncRNA encoding gene *AT5G01745* reversely localized at 3'-terminus of *SKS3*. Arrows show the translation direction; exons are in dark grey; untranslated region (UTR) is in light grey, and introns are shown as black lines.

Methods

RNA extraction and semi-quantitative RT-PCR

Total RNA was extract from 5-day-old seedlings, rossetta leaves, whole opening flowers and mature siliques from *Arabidopsis*, and cDNAs were synthesized by TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix (Transgene).

Primers

Primers utilized for semi-quantitative PCR: SKS3-F, TTTTCTCCATTTTCACTCACTGCT; SKS3-R, CTAATATGAT ATCCGATCCCGGTT; Actin-F, GTTAGCAACTGGGATGA TATGG; Actin-R, CAGCACCAATCGTGATGACTTGCCC.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

ORCID

Ke Zhou http://orcid.org/0000-0002-5883-2550

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