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Original Paper

A long 5′ UTR of the rice *OsMac1* mRNA enabling the sufficient translation of the downstream ORF

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Abstract Untranslated regions (UTRs) of mRNA are involved in many posttranscriptional regulatory pathways. The rice *OsMac1* mRNA had a 5′ UTR of more than 500 nucleotides (nt), containing a CU-rich region and three upstream open reading frames (uORFs) preceding the downstream ORF. The expected GFP-fusion protein was detected at the cell membrane, indicating the occurrence of translation of the downstream ORF. The 5′ UTR contained three splicing variants that were generated by alternative splicing. A reporter analysis using *β*-glucuronidase indicated that only the longest one, UTRc, showed a significant ability for the efficient translation of the downstream ORF, whereas other splicing variants showed low level of translational efficiency of the corresponding ORF. These results suggested that the additional 38-nt sequence unique to UTRc localized between the CU-rich region and the uORFs may be involved in the increased translational efficiency of the downstream ORF located after the long 5′ UTR.

Key words: Alternative splicing, membrane protein, rice (*Oryza sativa*), 5′ untranslated region, upstream open reading frame (uORF).

Gene expression is regulated precisely to maintain the homeostasis of the cell function. This process is achieved via multiple processes, including transcription, translation and posttranslational modification. In particular, translation is important for the determination of the accumulation of proteins. The initiation of the translation in eukaryotic cells involves a large number of factors, some of which are involved in the initial binding of the ribosome 40S subunit of the ribosome to the capped 5′ end of the mRNA, as well as in the binding of the initiator tRNA. After the initial binding, the complex containing the small ribosomal subunit scans the mRNA until it recognizes the first initiation codon, AUG. At this point, initiation factors are released and 60S subunit of the ribosome joins the complex, to start protein synthesis (Kozak 1999; Kozak 2007).

Recent studies revealed that translation is regulated by several mechanisms. Global regulation of translation involves the modulation of the activity of general factors, whereas mRNA-specific control is mediated by regulatory proteins or by noncoding RNAs. In particular, untranslated regions (UTRs) of mRNA are often involved in many posttranscriptional regulatory pathways that control mRNA localization, and stability and translation efficiency (Chatterjee and Pal 2009; Pesole et al. 2001).

Many 5′ UTRs of mRNAs contain multiple AUG codons, which suggests that the first-AUG rule is not followed in a remarkable fraction of mRNAs (Grillo et al. 2010; Pesole et al. 2001). The regulation of the translation of specific eukaryotic mRNAs is sometimes mediated by small upstream open-reading-frames (uORFs) that limit the access of the small subunit to a downstream ORF (Holcik and Pestova 2007). The yeast *GCN4* gene encodes the transcription factor Gcn4, the mRNA of which carries four uORFs in its 5′ leader sequence. The translation of GCN4 is activated by amino acid starvation. In this case, it is predicted that reduction in the levels of the ternary complex containing the methionyl initiator tRNA, eIF2, and GTP enables the bypassing of the start codons of uORFs by scanning ribosomes (Hinnebusch et al. 2004; Nielsen et al. 2004). Translational control by uORF located in the 5′ UTR has also been demonstrated, such as is the case for the *Arabidopsis* transcription factor bZIP11, the yeast AP1-like transcription factor Yap2, and the plant *S*-adenosylmethionine decarboxylase (AdoMetDC) mRNAs (Hanfrey et al. 2005; Rahmani et al. 2009; Vilela et al. 1999).

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We found a long 5′ UTR in a rice mRNA encoding a membrane protein. This 5′ UTR composed of three variants that were derived from the alternative splicing. Among them, one of the 5′ UTR showed significantly higher translational efficiency of the downstream ORF than those observed by the others. Here, we describe the characteristics of the 5′ UTR, and its effect on the translation of the downstream ORF.

Materials and methods

Plant materials

Rice (*Oryza sativa* L. cv. Nipponbare) was used as the wild-type plant, and was grown in a greenhouse.

PCR and real-time quantitative RT-PCR

Genomic DNA was prepared according to the method of Murray and Thompson (1980). PCR was performed using Blend-Taq thermostable DNA polymerase (Toyobo, Osaka, Japan). Total RNA was prepared from each tissue as described previously (Imamura et al. 2007). First-strand cDNA was synthesized from 1 μg of total RNA using the ReverTra Ace cDNA synthesis kit (Toyobo) with an oligo-dT(20) primer. Amplification of the entire cDNA of the AP004685 gene (termed *OsMac1*) was performed using primers, 5′-CACCGCACGCTACGCCTACG-3′ and 5′-AATGCCAGGGAGGTAAAAGGA-3′. The amplified fragments were cloned into pENTR^{TM}/D-TOPO (Invitrogen, Carlsbad, CA, USA). Real-time quantitative PCR was performed as described previously (She et al. 2010) using an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, CA, USA) with a SYBR Green real-time PCR mix (Toyobo). Primer sets (either 5′-CACATCTCCCTCAAGGATC-3′, 5′-ACATCTCCCTCAAGGTTG-3′, or 5′-TCACATCTCCCTCAAGCTA-3′ as a forward primer and 5′-CACGGTAGTATTCAACTGCTTG-3′ as a reverse primer) were used for measurement of the levels of the splice variants (UTRa, UTRb, and UTRc, respectively) of the *OsMac1* transcripts. The levels of β-glucuronidase (*GUS*) transcripts was measured using the primer set, 5′-GCCGATGCAGATATTCGTA-3′ and 5′-CCATCACTTCCTGATTATTGA-3′. The level of the *Actin1* mRNA (AK100267) was used as a positive control for the normalization of data. The primer set 5′-CCCTCCTGAAAGGAAGTACAGTGT-3′ and 5′-GTCCGAAGAATTAGAAGCATTTCC-3′ was used for detection of *Actin1* transcript.

Transient expression of the GFP fusion gene in onion epidermal cells

The region covering the ORF of the entire *OsMac1* transcript and that covering UTRc and the entire *OsMac1* ORF located after this sequence were amplified using the primer sets, 5′-CACCATGGAATTGGCAGAGC-3′ (orf-fw) and 5′-TTTCTCCCTAGTAATCCATC-3′ (orfns-rv), and 5′-CACCGCACGCTACGCCTACG-3′ (utr-fw) and orfns-rv, respectively, from the *OsMac1* cDNA containing UTRc as its 5′ UTR. The nucleotide sequence of orfns-rv was designed to remove the termination codon. The amplified fragment was entered into pENTR^{TM}/D-TOPO (Invitrogen) and was fused to the *GFP* gene using a pGWB5 plasmid (Nakagawa et al. 2007), via an LR clonase (Invitrogen) reaction. The *GFP* fusion gene was introduced into onion epidermal cells via particle bombardment (Bio-Rad, Richmond, CA, USA) and transient expression was detected according to von Arnim et al. (1998) using a confocal laser-scanning microscope (LSM 510 META; Carl Zeiss AG, Oberkochen, Germany).

Determination of GUS activity and estimation of translational efficiency

GUS activity was determined according to Jefferson et al. (1987). A plasmid harboring the *GUS* gene as a reporter gene was introduced into rice callus using the *Agrobacterium*-mediated transformation method (Hiei et al. 1994). Transgenic callus grown on 2N6 plates (Fujimura et al. 1985) supplemented with 50 mg l^{-1} Hygromycin B and 250 mg l^{-1} Claforan were transferred into R2 medium (Ling and Robinson 1997) supplemented with 50 mg l^{-1} Hygromycin B and 250 mg l^{-1} Claforan, and cultured for 10 days at 30°C, with medium exchange performed every 5 days. GUS activity was analyzed by staining for 15 min to 24 h at 37°C using a reaction solution containing 50 mM sodium phosphate buffer, pH 7.0, with 5% (v/v) methanol, 0.1% (v/v) Triton X-100 and 1 mM 5-bromo-4-chroro-2-indolyl-β-glucronide. To measure GUS activity, grown callus cells were homogenized in buffer (50 mM sodium phosphate buffer, pH 7.0, with 10 mM EDTA, 0.1% (v/v) Triton X-100, 0.1% (v/v) sodium *N*-lauroyl sarcosylate, 5 mM ($\pm$)-Dithiothreitol, and 20% (v/v) methanol). After cell debris was removed by centrifugation at 12,000 rpm for 5 min at 4°C, GUS activity was determined via reaction using 1 mM 4-methylumbelliferyl glucuronide as a substrate at 37°C for 30 min. Translational efficiency was estimated as the relative value of GUS activity relative to the level of the reporter mRNA, which was determined using real-time quantitative RT-PCR. The data were analyzed statistically using Student's *t* test.

Construction of reporter genes

The *GUS* gene, which was used as a reporter, was obtained from pBI221 (Jefferson et al. 1987), from which the coding region was amplified using primers that corresponded to the regions of the initiation and termination of the ORF (5′-CACCCTCGAGAGATTAGCCTTTTCAATTTC-3′

and 5′-TGAATTCCCGATCTAGTAACAT-3′), and then inserted into pENTR™/D-TOPO (Invitrogen) to generate pENTR-35S-GUS.

An expression plasmid containing each UTR followed by the *GUS* gene was obtained as follows: UTRa, UTRb, and UTRc were amplified from the corresponding cDNAs using the PCR primer set, 5′-CCCTCTAGAGACGCTACGCCTACGCGGGAG-3′ and 5′-GGGTCTAGATGCCAATTCCATTGTTCTCTC -3′. These sequences were inserted into the *Xba*I site preceding the *GUS* gene in pENTR-35S-GUS, after digestion of the amplified fragments with *Xba*I. Binary plasmids were produced using the resultant plasmids (pENTR-UTRa, pENTR-UTRb, and pENTR-UTRc, respectively) and pGWB1 (Nakagawa et al. 2007) via LR clonase reaction.

Results

A novel gene containing a long 5′UTR

We found a novel gene (AP004685; Os06g0726600) upstream of the gene that encodes the rice starch branching enzyme 1 (*Sbe1*), which is located on chromosome 6. This gene encoded a protein that exhibited local homology to the MAP-kinase activating protein and was named as *OsMac1*. The corresponding cDNA, which comprised 2,467 base-pairs, was registered in the database (acc. no. AK111844). The transcript of this gene had a long 5′ UTR composed of the 526 nt preceding the main ORF. This region contained a CU-rich region encompassing approximately 250 nt at the 5′ region, and three short ORFs (uORFs) at the 3′ region (Figure 1). These uORFs comprised 81, 78, and 72 nt, respectively, and encoded short peptides showing no homology to known proteins.

The *OsMac1* gene comprised 10 exons (Figure 2A). The predicted main ORF started from the region in the third exon, showing the 5′ UTR was encompassed with three exons (Figure 1). The CU-rich region was contained in the first exon. Three uORFs were contained in the region through the second and third exons. The nucleotide sequence of the corresponding cDNA showed existence of the different types of 5′ UTRs. Between the first and second exons, the event of alternative splicing was detected, resulting in formation of three variants of the *OsMac1* transcripts that were different in the structure of 5′ UTR (UTRa, UTRb, and UTRc, respectively) (Figure 2B). Among them, UTRa was the transcript whose nucleotide sequence corresponded to the cDNA registered as AK111844, while UTRb and UTRc contained 16-nt and 54-nt longer 5′ UTRs than that of UTRa (Figures 1B, 2B). The database contained another cDNA (AK112103) covering the 5′ end to the middle region of the main ORF, whose nucleotide sequence coincided with the 5′ UTR sequence in UTRc. The *OsMac1* gene was expressed ubiquitously. The transcript composed of UTRc was abundantly present 10 times more than those of other types of UTRs, both in leaves and immature seeds (Figure 2C). This result suggested that UTRc-type mRNA was the main product from *OsMac1*.

Translation of the main ORF of the OsMac1 transcript

The predicted protein encoded by the main ORF of the *OsMac1* contained a domain of unknown function 300 (DUF300) motif in the N-terminal region, which included six transmembrane regions (Figure 1A), suggesting that this protein is localized at the cell membrane. To determine the localization of this protein, we generated a fusion gene composed of the green fluorescent protein (*GFP*) gene, which was placed after the main ORF (Mac1-GFP), and driven by the CaMV 35S-promoter (Figure 3A). Transient expression of this gene in onion epidermal cells led to the detection of a fluorescence signal around the cell membrane region (Figure 3B), suggesting that this protein is localized at the membrane.

Next, we determined the localization of the protein produced from the transcript containing the fusion gene attached to the 5′ UTR of *OsMac1*. We constructed a fusion gene consisting of the main ORF of *OsMac1* and *GFP*, which was placed after UTRc (UTRcMac1-GFP; Figure 3A). The transient expression of this construct in onion epidermal cells led to detection of an obvious fluorescence signal around the cell membrane (Figure 3B), suggesting that the product of this gene is also localized at the cell membrane. This result indicates that the main ORF located downstream of this 5′ UTR is sufficiently translated.

UTRc achieved an enriched translation of the downstream ORF

We analyzed the efficiency of the translation of the ORF located after the 5′ UTRs. To estimate the translational efficiency according to the different 5′ UTRs, we constructed reporter genes including the *β*-glucuronidase (*GUS*) gene downstream from UTRa, UTRb, and UTRc and driven by the CaMV 35S-promoter (termed UTRa::GUS, UTRb::GUS, and UTRc::GUS, respectively; Figure 4A). These constructs were introduced into rice calli to determine GUS activity, which was compared with that of the control construct lacking the 5′ UTR (35S-GUS).

GUS activity was detected in the callus containing each of these reporter genes after 24 h incubation with that stain. Among them, significantly stronger GUS staining was detected after 15 min of incubation in the callus containing UTRc::GUS. In contrast, the callus harboring either UTRa::GUS or UTRb::GUS was weakly

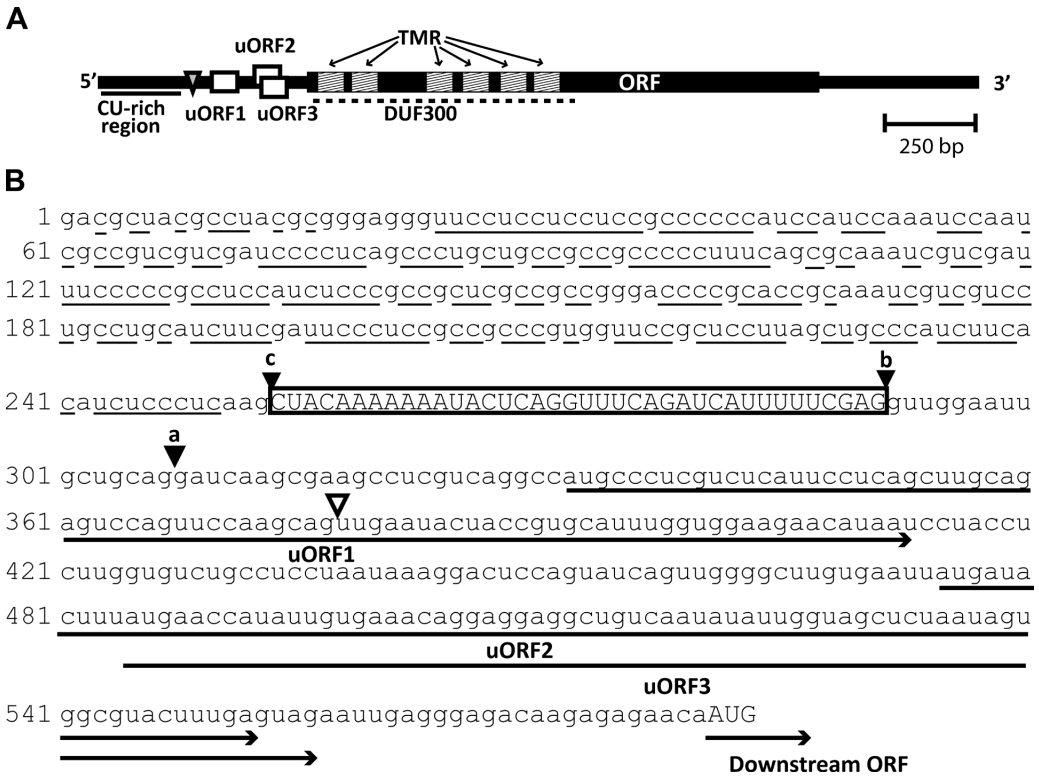

Figure 1. Structure of the *OsMac1* mRNA. (A) Schematic representation of the *OsMac1* mRNA. Open boxes and a filled box show the uORFs (uORF1, uORF2, and uORF3) and downstream ORF encoding a protein that is homologous to a MAP-kinase activation protein, respectively. The triangle indicates the region of the additional 38-nt sequence found in UTRc, and the hatched boxes in the downstream ORF show the location of the transmembrane regions (TMRs) located in the DUF300 domain included in the predicted protein. (B) Nucleotide sequence of the UTRc of the *OsMac1* mRNA. The CU-rich sequences in the 5′ region are underlined. The open box indicates the additional 38-nt sequence. uORFs and the downstream ORF are indicated by arrows placed below the nucleotide sequence. Regions between filled triangles a and c, and those between b and c, lack UTRa and UTRb, respectively. The open triangle shows the position of the splicing site located between the second and third exons.

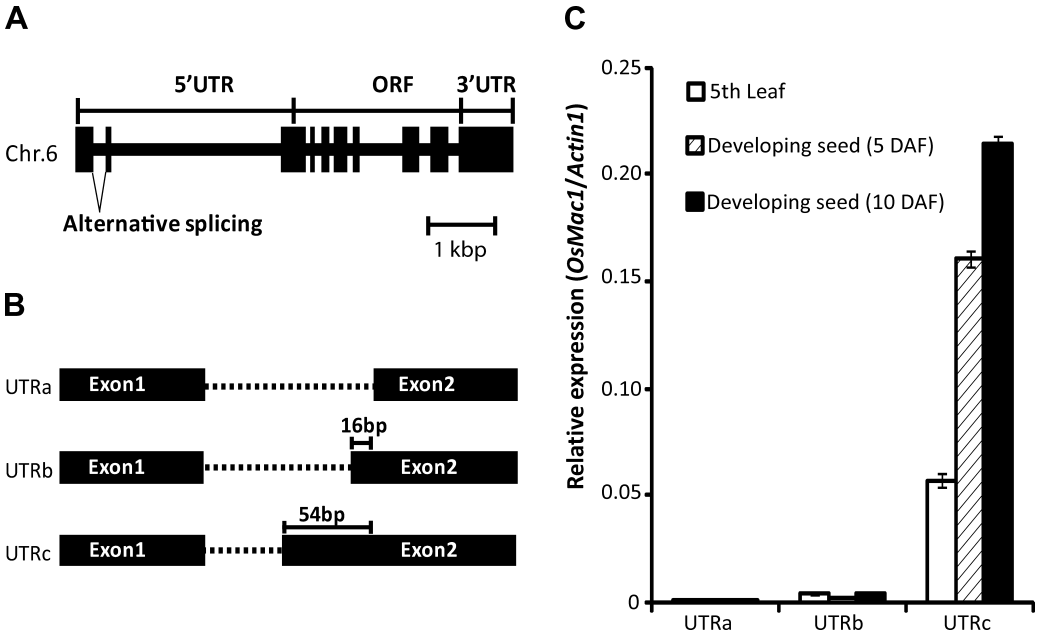

Figure 2. Alternative splicing in the 5′ UTR of the *OsMac1* mRNA. (A) Genomic structure of the *OsMac1* gene. Filled boxes indicate exons. The site of alternative splicing is shown. (B) Schematic representation of the alternative splicing of UTRa, UTRb, and UTRc. Spliced regions are indicated by broken lines. (C) Expression of each variant of the *OsMac1* mRNA in the 5th leaf and in developing seeds at 5 days after flowering (DAF) and 10 DAF. The level of these transcripts is shown as a relative value to that observed for *Actin1*; these level were determined using real-time quantitative RT-PCR. Error bars indicate SD (n=5).

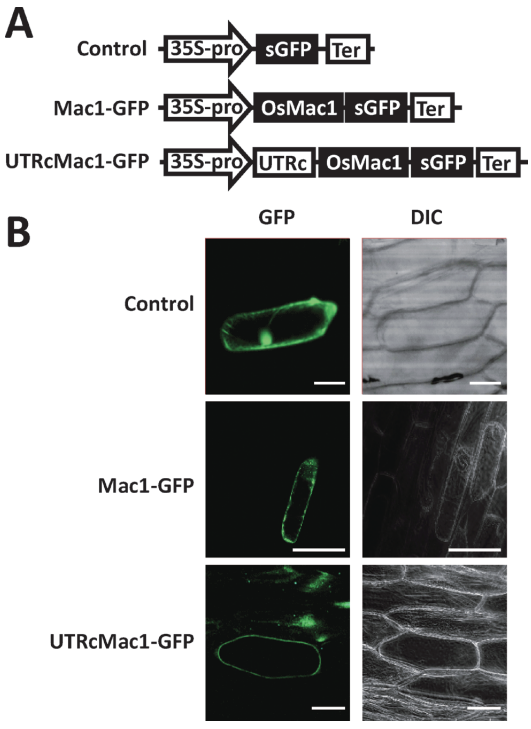

Figure 3. Translation of the main ORF of the *OsMac1* mRNA. (A) Schematic representation of the structure of the expression plasmids, which were constructed for transient expression in onion epidermal cells. Mac1-GFP indicates the fusion gene consisting of the entire *OsMac1* sequence with sGFP driven by the CaMV 35S promoter (35S-pro), and UTRcMac1-GFP contained the fusion gene placed after UTRc. The control plasmid contained sGFP. Ter, NOS terminator. (B) Detection and subcellular localization of the translated OsMac1-GFP fusion proteins in onion epidermal cells. Fluorescence images of GFP and differential interference contrast macroscopic images (DIC) are shown. Genes were introduced using microprojectile methods. Scale bar = 100 μm.

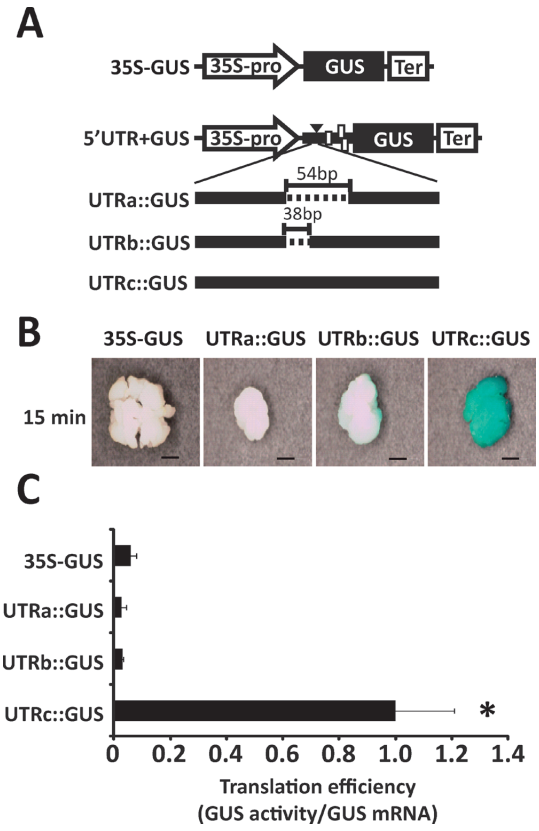

Figure 4. Translational efficiency of the UTRs. (A) Structure of the reporter *GUS* genes placed downstream of the UTRs. The position of the additional 38-nt sequence is indicated by a triangle. The regions around this sequence in UTRa, UTRb, and UTRc are highlighted below. Broken lines indicate the nucleotides missing in UTRa and UTRb, via the alternative splicing. 35S-pro, CaMV 35S-promoter; ter, NOS-terminator. (B) GUS-activity staining in the callus cells harboring each gene. The upper panels show the cell lines after 15 min of staining. Scale bar=500 μm. (C) Translational efficiency is shown as the values of GUS activity relative to the amount of its mRNA in the transformed callus cells. The translational efficiency of UTRc::GUS was set at 1.0. Error bars indicate the SD (n=5). An asterisk indicates a significant difference between the translational efficiency of the individual UTR and the 35S-GUS at $P<0.05$.

stained for GUS to a lesser extent than that detected in the callus transformed by 35S-GUS (Figure 4B). The difference in GUS activity depended on the gene introduced.

We determined the efficiency of translation of the downstream ORF, which was estimated as the ratio of the GUS activity to the amount of these mRNAs in the cell. The callus transformed with UTRc::GUS exhibited a translational efficiency that was ~15-fold higher than that detected in the callus harboring the control gene. The transformants containing UTRa::GUS or UTRb::GUS exhibited a very low translational efficiency, which was approximately half of that detected in 35S-GUS (Figure 4C). A similar amount of mRNA was detected in all callus lines using real-time quantitative RT-PCR (qRT-PCR), suggesting that nearly equal transcription occurred from each fusion gene. These results indicate that UTRc possesses a specific region that increases the translational efficiency of the downstream ORF located after the long 5′ UTR. As UTRc was 38 nt longer than UTRb (Figure 1), it is expected that this additional 38-nt region is involved in the efficiency of the translation of the downstream ORF. The nucleotide sequence of the additional 38-nt sequence predicted to form a stem-loop structure (Figure 5). This sequence showed no homology to any known *cis* elements involved in translation.

Discussion

The rice *OsMac1* mRNA possesses a long 5′ UTR preceding the main ORF, which includes a CU-rich region and three uORFs that are predicted to affect on the translational efficiency of the downstream ORF (Figure 1). The protein encoded by the downstream ORF was localized at the cell membrane, as its GFP fusion protein was detected at the cell membrane when it was

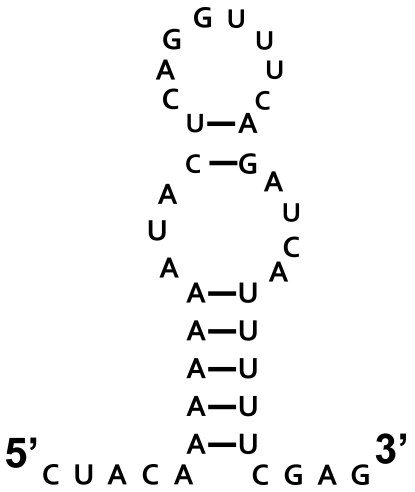

Figure 5. A predicted stem–loop structure of the additional 38-nt sequence.

expressed without the long 5′ UTR. *In vivo* translation of the RNA, which was transcribed from a chimeric construct that encoded the GFP fusion protein placed after the long 5′ UTR, resulted in localization of the expected protein at the cell membrane (Figure 3). This finding suggests that the 5′ UTR of the *OsMac1* mRNA allows the efficient translation of the downstream ORF.

The three variants of the 5′ UTR (UTRa, UTRb, and UTRc) are derived by alternative splicing between the first and second exons (Figure 1). Among them, UTRc was detected abundantly in rice cells (Figure 2). UTRa and UTRb contained 5′ UTRs that were 54 nt and 38 nt shorter than that of UTRc. Among these UTRs, UTRc led to efficient translation of the downstream ORF, whereas UTRa and UTRb showed low level of translational efficiency (Figure 4). UTRc increased the translation efficiency of the mRNA encoding the GUS protein, and the 38-nt sequence was uniquely contained in UTRc. A stem–loop structure was predicted to form in this region (Figure 5), although this sequence showed no homology to any enhancer sequence reported previously.

uORF is known as a translational negative regulator. It has been shown that translational repression occurs in response to environmental stimuli and is mediated by the short peptide encoded by the uORF located in the 5′ UTR of the *Arabidopsis* bZIP11 transcript (Rahmani et al. 2009). Some mRNAs contain an unusual oligopyrimidine tract at their transcriptional start site, termed 5′ terminal oligopyrimidine tract (5′ TOP), which is involved in the growth-dependent translational regulation (Crosio et al. 2000; Jefferies et al. 1997). It has been shown that the RNA-binding protein HuR and the polypyrimidine-tract-binding protein (PTB) promote the translation of HIF-1α, and that they bind to the RNA motifs that are predicted to form a specific secondary structures (López de Silanes et al. 2004; Fernandez et al. 2005; Galbán et al. 2008). All of the *OsMac1* UTRs contained three uORFs and the CU-rich region (Figure 1). We presume that the 38 nt region unique to UTRc localized between these regions gives some significance to express different translational efficiency of the downstream ORF among the UTRs.

Models of Cap-independent mechanisms of translation initiation have been proposed. In this case, it is suggested that extensive protein–protein and protein–RNA interactions are required to bring together the translation factors, tRNAs, ribosomes, and mRNA (Gallie 2002; Thiébeauld et al. 2007). The 68 nt 5′ leader (called Ω) sequence of tobacco mosaic virus genome RNA functions as a translational enhancer. The Ω sequence has a primary structure caused by two elements, a direct repeat of 8 nt and a CAA-rich region, which are responsible for translation enhancement (Gallie and Walbot 1992; Schmitz et al. 1996; Wells et al. 1998). The 5′ UTR of the *OsMac1* mRNA comprised more than 500 nt, and no homology was found to the Ω sequence. The 5′ noncoding region of the mRNA that utilizes a striking complementarity to 18S rRNA facilitates a novel form of translation initiation referred to as ribosome shunting, in which 40S ribosomes bind the cap and bypass large segments of the mRNA to reach the initiation codon. It has been suggested that ribosome shunting may involve either specific RNA structural feature or a prokaryotic-like interaction between mRNA and rRNA (Yueh and Shneider 2000). In the *OsMac1* mRNA, there might be a similar mechanism for translation of the downstream ORF.

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References

Chatterjee S, Pal JK (2009) Role of 5′- and 3′-untranslated regions of mRNAs in human diseases. *Biol Cell* 101: 251–262

Crosio C, Boyl PP, Loreni F, Pierandrei-Amaldi P, Amaldi F (2000) La protein has a positive effect on the translation of TOP mRNAs *in vivo*. *Nucleic Acids Res* 28: 2927–2934

Fernandez J, Yaman I, Huang C, Liu H, Lopez AB, Komar AA, Caprara MG, Merrick WC, Snider MD, Kaufman RJ, Lamers WH, Hatzoglou M (2005) Ribosome stalling regulates IRES-mediated translation in eukaryotes, a parallel to prokaryotic attenuation. *Mol Cell* 17: 405–416

Fujimura T, Sakurai M, Akagi H, Negishi T, Hirose A (1985) Regeneration of rice plants from protoplasts. *Plant Tissue Cult Lett* 2: 74–75

Galbán S, Kuwano Y, Pullmann R Jr, Martindale JL, Kim HH, Lal A, Abdelmohsen K, Yang X, Dang Y, Liu JO, Lewis SM, Holcik M, Gorospe M (2008) RNA-binding proteins HuR and PTB promote the translation of hypoxia-inducible factor 1*α*. *Mol Cell Biol* 28: 93–107

Gallie DR, Walbot V (1992) Identification of the motifs within the tobacco mosaic virus 5′-leader responsible for enhancing translation. *Nucleic Acids Res* 20: 4631–4638

Gallie DR (2002) Protein-protein interactions required during translation. *Plant Mol Biol* 50: 949–970

Grillo G, Turi A, Licciulli F, Mignone F, Liuni S, Banfi S, Gennarino VA, Horner DS, Pavesi G, Picardi E, Pesole G (2010) UTRdb and UTRsite (RELEASE 2010): a collection of sequences and regulatory motifs of the untranslated regions of eukaryotic mRNAs. *Nucleic Acids Res* 38(Database issue): D75–D80

Hanfrey C, Elliott KA, Franceschetti M, Mayer MJ, Illingworth C, Michael AJ (2005) A dual upstream open reading frame-based autoregulatory circuit controlling polyamine-responsive translation. *J Biol Chem* 280: 39229–39237

Hiei Y, Ohta S, Komari T, Kumashiro T (1994) Efficient transformation of rice (*Oryza sativa* L.) mediated by Agrobacterium and sequence analysis of the boundaries of the T-DNA. *Plant J* 6: 271–282

Hinnebusch AG, Asano K, Olsen DS, Phan L, Nielsen KH, Valásek L (2004) Study of translational control of eukaryotic gene expression using yeast. *Ann NY Acad Sci* 1038: 60–74

Holcik M, Pestova TV (2007) Translation mechanism and regulation: old players, new concepts. Meeting on translational control and non-coding RNA. *EMBO Rep* 8: 639–643

Imamura T, Kusano H, Kajigaya Y, Ichikawa M, Shimada H (2007) A rice dihydrosphingosine C4 hydroxylase (DSH1) gene, which is abundantly expressed in the stigmas, vascular cells and apical meristem, may be involved in fertility. *Plant Cell Physiol* 48: 1108–1120

Jefferies HB, Fumagalli S, Dennis PB, Reinhard C, Pearson RB, Thomas G (1997) Rapamycin suppresses 5'TOP mRNA translation through inhibition of p70s6k. *EMBO J* 16: 3693–3704

Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO J* 6: 3901–3907

Kozak M (1999) Initiation of translation in prokaryotes and eukaryotes. *Gene* 234: 187–208

Kozak M (2007) Some thoughts about translational regulation: forward and backward glances. *J Cell Biochem* 102: 280–290

Ling MM, Robinson BH (1997) Approaches to DNA mutagenesis: an overview. *Anal Biochem* 254: 157–178

López de Silanes I, Zhan M, Lal A, Yang X, Gorospe M (2004) Identification of a target RNA motif for RNA-binding protein HuR. *Proc Natl Acad Sci USA* 101: 2987–2992

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res* 8: 4321–4325

Nakagawa T, Kurose T, Hino T, Tanaka K, Kawamukai M, Niwa Y, Toyooka K, Matsuoka K, Jinbo T, Kimura T (2007) Development of series of gateway binary vectors, pGWBs, for realizing efficient construction of fusion genes for plant transformation. *J Biosci Bioeng* 104: 34–41

Nielsen KH, Szamecz B, Valásek L, Jivotovskaya A, Shin B-S, Hinnebusch AG (2004) Functions of eIF3 downstream of 48S assembly impact AUG recognition and *GCN4* translational control. *EMBO J* 23: 1166–1177

Pesole G, Mignone F, Gissi C, Grillo G, Licciulli F, Liuni S (2001) Structural and functional features of eukaryotic mRNA untranslated regions. *Gene* 276: 73–81

Rahmani F, Hummel M, Schuurmans J, Wiese-Klinkenberg A, Smeekens S, Hanson J (2009) Sucrose control of translation mediated by an upstream open reading frame-encoded peptide. *Plant Physiol* 150: 1356–1367

Schmitz J, Prüfer D, Rohde W, Tacke E (1996) Non-canonical translation mechanisms in plants: efficient *in vitro* and *in planta* initiation at AUU codons of the tobacco mosaic virus enhancer sequence. *Nucleic Acids Res* 24: 257–263

She K-C, Kusano H, Koizumi K, Yamakawa H, Hakata M, Imamura T, Fukuda M, Naito N, Tsurumaki Y, Yaeshima M, Tsuge T, Matsumoto K, Kudoh M, Itoh E, Kikuchi S, Kishimoto N, Yazaki J, Ando T, Yano M, Aoyama T, Sasaki T, Satoh H, Shimada H (2010) A novel factor FLOURY ENDOSPERM2 is involved in regulation of rice grain size and starch quality. *Plant Cell* 22: 3280–3294

Thiébeauld O, Pooggin MM, Ryabova LA (2007) Alternative translation strategies in plant viruses. *Plant Viruses* 1: 1–20

Vilela C, Ramirez CV, Linz B, Rodrigues-Pousada C, McCarthy JEG (1999) Post-termination ribosome interactions with the 5′ UTR modulate yeast mRNA stability. *EMBO J* 18: 3139–3152

von Arnim AG, Deng X-W, Stacey MG (1998) Cloning vectors for the expression of green fluorescent protein fusion proteins in transgenic plants. *Gene* 221: 35–43

Wells DR, Tanguay RL, Le H, Gallie DR (1998) HSP101 functions as a specific translational regulatory protein whose activity is regulated by nutrient status. *Genes Dev* 12: 3236–3251

Yueh A, Schneider RJ (2000) Translation by ribosome shunting on adenovirus and *hsp70* mRNAs facilitated by complementarity to 18S rRNA. *Genes Dev* 14: 414–421