Analysis of five novel putative constitutive gene promoters in transgenic rice plants

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Supplementary Materials

Supplementary data are available at *JXB* online.

Supplementary Table S1. Microarray data for seven constitutive rice genes in 14-day old leaves and roots and early stage flowers.

Supplementary Table S2. Primers used in this study for RT-PCR and promoter isolation.

Supplementary Fig. S1. Structure of the *SCP1* 5' UTR.

Supplementary Fig. S2. Potential regulatory elements in the promoter regions of the eight constitutive genes analyzed in this study.

Supplementary Table S1. Microarray data for seven constitutive rice genes in 14-day old leaves and roots and early stage flowers.

Gene	Accession No.[a]	Intensity[b]					
		Leaf		Root		Flower	
		repeat1	repeat2	repeat1	repeat2	repeat1	repeat2
APX	**AK068430**	30,785	34,118	34,445	36,445	44,140	41,005
SCP1	**AK101133**	30,168	30,313	51,879	56,893	32,864	36,316
PGD1	**AK065920**	20,716	23,222	43,532	50,458	32,756	33,151
R1G1B	**AF503583**	24,641	34,470	48,095	49,468	8,990	8,760
EIF5	**AK060387**	25,468	23,663	42,139	39,806	37,727	42,025
OsCc1	**AK060267**	20,643	20,252	34,386	33,154	25,736	24,631
Act1	**AK100267**	24,925	29,283	50,056	42,771	41,418	40,581

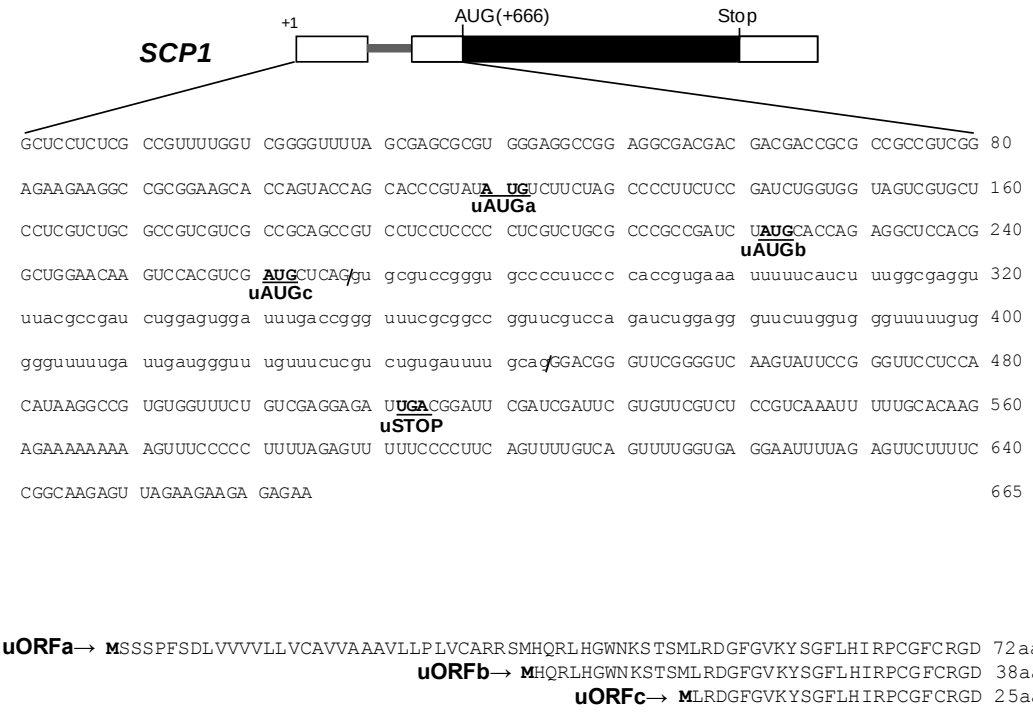
[a]Accession numbers for full-length cDNA sequences of the corresponding genes. [b]Numbers represent the spot intensity values from microarray analysis of two independent biological replicates.

Supplementary Table S2. Primers used in this study for RT-PCR and promoter isolation

Gene	Specificity	Accession No.[a]	Product Size (bp)	Primer Sequence	
				Forward	Reverse
APX	gene	AK068430	237	5'- GACCTCTAGACCGCCGTATT -3'	5'- GCCAACCACTCGCAATCCAA -3'
SCP1	gene	AK101133	240	5'- TCGCTGCCTACGCCAACATC -3'	5'- TCGCCGAACTAGCAGGTGAG -3'
PGD1	gene	AK065920	238	5'- CGGTGAGCTAGCGAGGATCT -3'	5'- CCGGTAGGAGTCGAAGTACG -3'
R1G1B	gene	AF503583	250	5'- CTTCTCGATTGCCGTGTGCT -3'	5'- GCAAGTCTCAAGCTCTCAAT -3'
EIF5	gene	AK060387	168	5'- GATCTGCGCTCTGAAGGATA -3'	5'- AACCGCAAGATGGAACAACG -3'
OsCc1	gene	AK060267	127	5'- ACTCTACGGCCAACAAGAAC -3'	5'- CTCCTGTGGCTTCTTCAACC -3'
Act1	gene	AK100267	124	5'- ATGGTGTCAGCCACACTGTC -3'	5'- TAACCACGCTCCGTCAGGAT -3'
OsUbi1	gene	AK121590	187	5'- ATGGAGCTGCTGCTGTTCTA -3'	5'- TTCTTCCATGCTGCTCTACC -3'
gfp	gene	CS478164	141	5'- CAGCACGACTTCTTCAAGTCC -3'	5'- CTTCAGCTCGATGCGGTTCAC -3'
APX	promoter	AK068430	1673	5'- GTAAGGTGACATGGCATATC -3'	5'- CCAATCCGAATCAATCAATC -3'
SCP1	promoter	AK101133	1853	5'- TTGACTTTTTCTGCGAAGAA -3'	5'- TAACTCTTGCCGGAAAAGAA -3'
PGD1	promoter	AK065920	1891	5'- TAGATATGCCGAACATGACC -3'	5'- GCAGATAGATGCACCAAATG -3'
R1G1B	promoter	AF503583	1799	5'- ATAGCTGTTGTACTGATGTC -3'	5'- TCTCTCGCAGTATTACCAAC -3'
EIF5	promoter	AK060387	1837	5'- TTGTTCCACCTCATCATTAA -3'	5'- CAACCTGCCACCAACAACAA -3'
OsCc1	promoter	AK060267	1882	5'- TCGAAGGTAGGCTGCAGTTCT -3'	5'- CCCGGGGGAGCCTCCGAGAA -3'
Act1	promoter	AK100267	1263	5'- AGCTAGCATACTCGAGGTCAT -3'	5'- TATCCTCGGCGTCAGCCATC -3'
ZmUbi1	promoter	DQ141598	1973	5'- CGGTCGTGCCCCTCTCTAGA -3'	5'- CTGCAGAAGTAACACCAAACA -3'

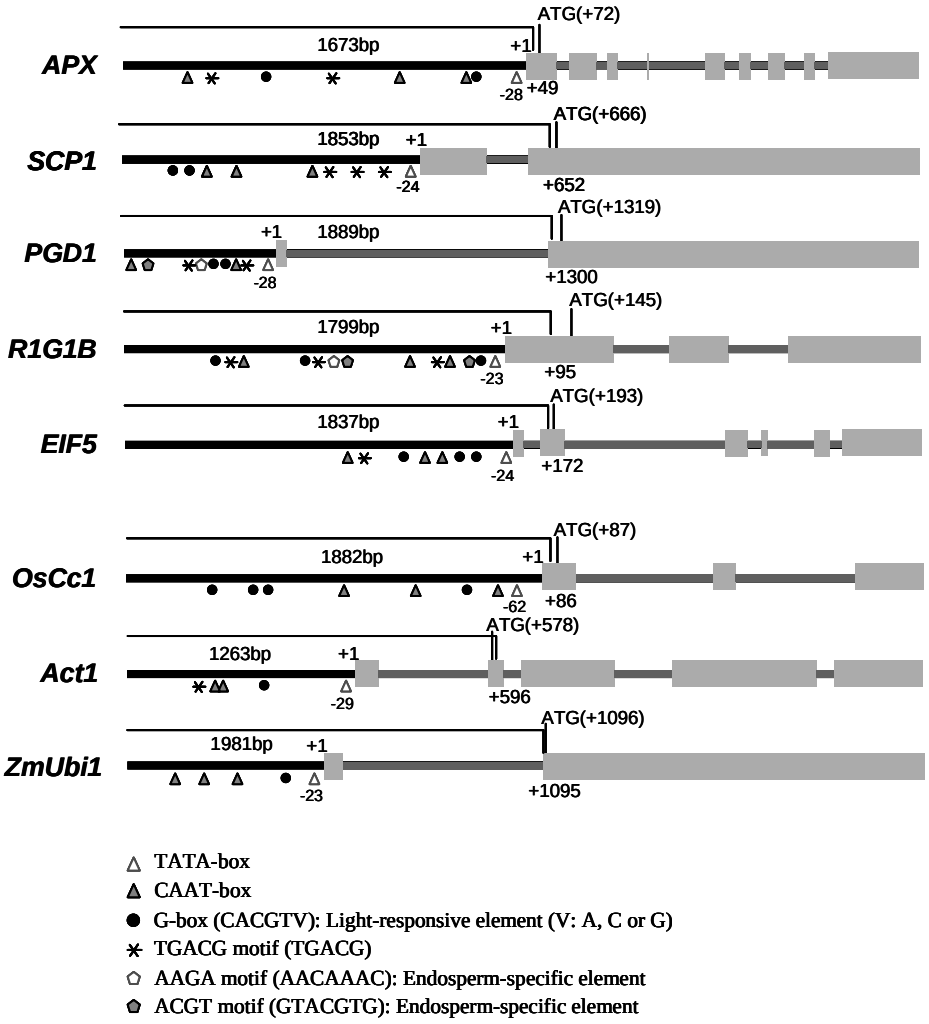
Forward and reverse primers for promoter isolation have additional 12mers, AAAAAGCAGGCT and AGAAAGCTGGGT, at their 5'-ends, respectively. The first PCR products for promoters were re-amplified for cloning with the adaptor primers attB1 5'-GGGGACAAGTTTGTACAAAAAAGCAGGCT-3'and attB2 5'-GGGGACCACTTTGTACAAGAAAGCTGGGT-3'.

Fig. S1



Supplementary Fig. S1. Structure of the *SCP1* 5' UTR. The *SCP1* transcription initiation site is indicated by +1. The principal AUG is located at +666. Three underlined upstream AUGs (uAUGs) are located at positions +120, +222 and +261 and three upstream open reading frames (uORFa, b and c; shown in the lower panel) consist of 72, 38 and 25 codons, respectively. The upstream STOP (uSTOP) codon is located at +512. Exons and 5' or 3' UTRs are shown as filled and white boxes, respectively. Introns are indicated by grey lines between boxes. Upper and lower case letters represent the exon sequences (489 bp long) and intron sequences (176 bp long), respectively. The forward slashes indicate exon-intron boundaries.

Fig. S2



Supplementary Fig. S2. Potential regulatory elements in the promoter regions of the eight constitutive genes analyzed in this study. The TATA and CAAT sequences are indicated by white and grey triangles, respectively. The TATA start positions are marked below each white triangle. G-box, filled circle; TGACG, star; AAGA and ACGT motif, white and grey pentagon, respectively. Bold lines, 5'upstream promoter region; filled boxes, exons; lines between the boxes, introns. The lengths of all boxes were drawn to scale. The transcriptional start site and the translational start codon are denoted by +1 and ATG, respectively.