Supplemental Material and Methods

RNA isolation from *Nicotiana benthamiana* and Reverse Transcription reaction

Four days after *N. benthamiana* infiltration, leaf samples (100 mg) were taken and immediately frozen in liquid nitrogen. Tissue was grounded using a Mixer Mill MM400 (Retsch, Haan, Germany). RNA was isolated from the grounded tissue using TriFast Solution (Peqlab, Erlangen, Germany) according to the manufacturer's protocol. 1 µg of RNA was used for the Reverse Transcription reaction. The RNA was premixed with 0,5 µl random hexamer primer (1µg/ml) in a volume of 36 µl and incubated for 10 min at 80°C, 10 min at 60°C, 10 min at 40°C and 10 min at 20°C. The RNA-primer mixture was placed on ice and mixed with 25 nmol dNTPs, 2u RNasin, and 200u MMLV Reverse Transkriptase (Promega, Mannheim, Germany) in a reaction volume of 50 µl. The reaction mixtures were incubated for 10 min at 37°C, 60 min at 42°C and for 10 min at 80°C and finally placed on ice. 4 µl of the cDNA was used as a template in a PCR reaction using the Phusion Polymerase (NEB, Frankfurt, Germany) according to the manufacturer's protocol.

Amplification and cloning of *Arabidopsis* PATs

All Arabidopsis PATs were amplified using either cDNAs from different *Arabidopsis thaliana* (Col-0 wild type) tissues (root, shoot or pollen from Col-0 wild type), an *Arabidopsis* cDNA two-hybrid library (Kim et al., 1997) or plasmids which contain isolated reading frames (provided either from RIKEN or from the Arabidopsis Resource Centre) (see Table 1). All AtPATs were inserted into a pGPTVII backbone plasmid using respective restriction sites in front of a GFP reading frame, which results in a PATx.GFP fusion.

AtPAT20 was not amplifiable from the available cDNA sources. Therefore, in contrast to the other PATs a genomic fragment (including the introns) of AtPAT20 was amplified from *Arabidopsis* genomic DNA (gDNA) and fused to GFP (Figure 1A). This gDNA-GFP fusion was used for initial localization analysis and expressed in *N. benthamiana* via *A. tumefaciens* mediated infiltration. Although using the genomic fragment, the protein fusion was well expressed (not shown), implicating that the introns are efficiently and correctly spliced out. To obtain a cDNA, RNA was extracted from the transiently expressing *N. benthamiana* leaves and AtPAT20 was re-amplified by RT-PCR. The obtained DNA fragment was clearly smaller than the genomic fragment and of the expected size showing correct splicing (Figure 1B). Moreover, sequencing of the re-amplified AtPAT20 cDNA fragment proofed the correct splicing and structure of the annotated reading frame.

Amplification and cloning of AtCNX1, AtTPK1, AtGNT1

Arabidopsis CNX1, TPK1, GNT1 (AGI numbers see Table 1) were amplified using either cDNA (from Col-0 wt plants) or plasmids (see Table 1). All reading frames were inserted into a pGPTVII backbone plasmid using respective restriction sites either in front of a mCherry (TPK1, GNT1) or behind the mCherry reading frame (CNX1), respectively. Expression is driven by the mannopine synthase (MAS) promoter.

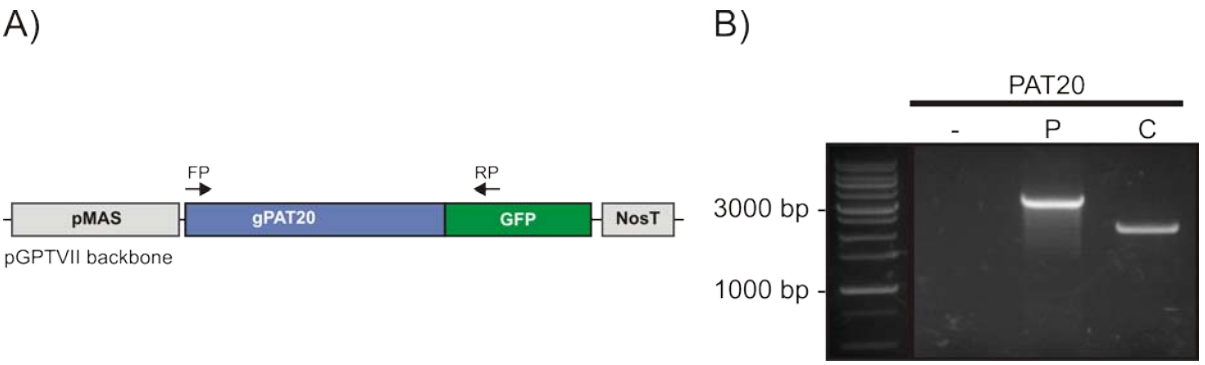

Figure 1:

A) Genomic fragment of PAT20 (gPAT20) from Arabidopsis was introduced into the pGPTVII plasmids and fused to the N-terminus of the GFP reading frame. (pMAS = MAS promoter; NosT = Nos terminator)

B) A PAT20 specific forward primer (FP) and a reverse primer (RP), which binds to the GFP reading frame (see A), were used to amplify PAT20 either from the plasmid generated (P = plasmid input) or from the cDNA (C = cDNA output) obtained from *Nicotiana benthamiana* leaves which were infiltrated with *A. tumefaciens* cells transformed with the respective plasmid. **–** = water control.

Table 1: Primers used in this work

Primer	**primer sequence 5' → 3'** (restriction sites underlined)	**construct**	**Template**
PAT1for	AAAATCTAGAAAAATGTCTTCTCAGAATCTTGAACG	AtPAT01	U87166[1]
PAT1rev	TTTTCTCGAGCCGTTCTCTAGCTTCGGAATC		
PAT2for	TTTTTCTAGAAAAATGGGGAGGAAGAAAAGCTG	AtPAT02	pollen cDNA[3]
PAT2rev	AAAAGGTACCCCTCGATCTCACATCAATGC		
PAT3for	TTTTCTAGATGAAAAATGCAGAGGGAGAGAATGAGC	AtPAT03	S63794[1]
PAT3rev	AAAACCCGGGCTTGGGCAAGTCTAGTTGAGATG		
PAT4for	AAAATCTAGAAAAATGGCTTGGAATGAGACCAAAC	AtPAT04	cDNA library
PAT4rev	AAAACTCGAGTTTACGAAGCATTGGTGAAGTGG		
PAT5for	TTTTTCTAGAAAAATGTTAGATTTGCAGCCGTCAG	AtPAT05	root cDNA
PAT5rev	TTTTCCCGGGTAACCGCCCTATACCAGTTCC		
PAT6for	TTTTACTAGTTGAAAAATGTATGTGGTGACGCCTCC	AtPAT06	U10343[1]
PAT6rev	AAAACTCGAGAGCTCTTCTTGCTGATAAGGC		
PAT7for	TTTTTCTAGAAAAATGTATGTAGTGCCTCCGCC	AtPAT07	pdz24089[2]
PAT7rev	AAAACCCGGGTGTAGGCCGGTTCTCCGTC		
PAT8for	TTTTGGATCCAAAATGACACAACGGGTATTCCAAG	AtPAT08	cDNA library
PAT8rev	AAAACCCGGGTCCCCTTCCATCCTTTGCTG		
PAT9for	TTTTACTAGTTGAAAAATGGCTGGACGGGTCTTCG	AtPAT09	U18817[1]
PAT9rev	AAAACTCGAGCCGTCCCTCCTCTGCAG		

PAT10for	TTTGGATCCAAAATGGGCGTTTGTTGCCCTTTC	AtPAT10	cDNA library
PAT10rev	AAAACCCGGGGCAGCAGCGACATTTCAAC		
PAT11for	TTTTCTAGAAAAATGGAAGATTCTTCCCAGGGG	AtPAT11	cDNA library
PAT11rev	AAAACCCGGGTGTCTTATGTCTCTTCCTCAAG		
PAT12for	TTTTTCTAGAAAAATGAACCTTTTCCGGTTCTGC	AtPAT12	U67062[1]
PAT12rev	AAAACCCGGGAGGGTCGATGTCAGAACAAG		
PAT13for	AAAATCTAGAAAAATGGCGTGGAACGTGTTCAAG	AtPAT13	U09586[1]
PAT13rev	TTTTCCCGGGTAGAGACTGAAGCGGCTCTG		
PAT14for	TTTTTCTAGAAAAATGCATAGATCTGGTACAACAATG	AtPAT14	U19236[1]
PAT14rev	AAAACCCGGGCTGGGAATCAAAGTCGGGTTTC		
PAT15for	TTTTTCTAGAAAAATGGGTTTCGTGTATTACGTCAC	AtPAT15	cDNA library
PAT15rev	AAAACCCGGGGCTGTCTCTCGATGCAGAAAAG		
PAT16for	TTTTTCTAGATGAAAAATGAAACGGAAAGGAGTCGG	AtPAT16	U12747[1]
PAT16rev	AAAACCCGGGATGTTTTGTTTCAGATGAATCAGG		
PAT17for	TTTTCTAGAAAAATGGCGGTACAGTGGCTTTTG	AtPAT17	pda19162[2]
PAT17rev	AAAACCCGGGTTCCGATTTTCGTTTTGATTTGTTG		
PAT18for	TTTTGCTAGCAAAATGTGTTACTGCTTTGTGCAG	AtPAT18	cDNA library
PAT18rev	AAAACCCGGGTCTTGGTGATGCTTCACTTCC		
PAT19for	AAAATCTAGAAAAATGGTGAGGAAACATGGTTGG	AtPAT19	U25382[1]
PAT19rev	TTTTCCCGGGCTTGCGGGTACCTACAGG		
PAT20for	TTTTGCTAGCAAAATGGTGAGGAAACACGGTTGG	AtPAT20	genomic DNA
PAT20rev	AAAACCCGGGCTTGATGTTAGAACCGGTTTGG		
PAT21for	TTTTTCTAGAAAAATGGCGAGAAGACATGGATGG	AtPAT21	PENTR221 AT2G66340[1]
PAT21rev	AAAACCCGGGATGGAATCTAGTAGATAAATGTCTGTG		
PAT22for	TTTTGGATCCAAAATGAGGAAACATGGATGGCAG	AtPAT22	cDNA library
PAT22rev	AAAACCCGGGTCTTGATTTGTGGATGTTTCTAG		
PAT23for	TTTTACTAGTTGAAAAATGGACTCATCAGAGATCGAAG	AtPAT23	pollen cDNA[3]
PAT23rev	AAAACTCGAGGAGAATTGAAGACGGGACAAC		
PAT24for	TTTTCTCGAGAAAATGTCATCGGAGATTGAGGTG	AtPAT24	plasmid[4]
PAT24rev	AAAACCCGGGTGGAGATACAACAGGTCGGG		
GFP5'rev	CAACAAGAATTGGGACAACTCC	AtPAT20	*N. benthamiana* cDNA
CNX1for	TTTTACTAGTAGACAACGGCAACTATTTTCCG	CNX1 (At5g61790)	cDNA library
CNX1rev	AAAACCCGGGCTAATTATCACGTCTCGGTTGC		
TPK1for	TTTGGATCCATGTCGAGTGATGCAGCTC	TPK1 (AT5G55630)	plasmid[5]
TPK1rev	TTTCTCGAGCCTTTGAATCTGAGACGTGG		
GNT1for	TTTTTCTAGAAAAATGGCGAGGATCTCGTGTG	GNT1 (At4g38240)	plasmid[6]
GNT1rev	AAAACCCGGGGGAATTTCGAATTCCAAGCTGC		

[1] Uxxxxx, Sxxxxx, PENTRxxx, clones obtained from the Arabidopsis stock centre

[2] pdaxxxxx or pdzxxxxx, clones obtained from RIKEN

[3] gift form D. Becker

[4] plasmid gift from P. Hemsley and C. Grierson

[5] plasmid previously used; **Batistic O, Waadt R, Steinhorst L, Held K, Kudla J** (2010) CBL-mediated targeting of CIPKs facilitates the decoding of calcium signals emanating from distinct cellular stores. Plant J **61:** 211-222

Constructs published previously:

Construct	Reference
CBL1n.OFP	**Batistic O, Waadt R, Steinhorst L, Held K, Kudla J** (2010) CBL-mediated targeting of CIPKs facilitates the decoding of calcium signals emanating from distinct cellular stores. Plant J **61:** 211-222

Supplemental References:

Kim J, Harter K, Theologis A (1997) Protein-protein interactions among the AUX-IAA proteins. Proc Natl Acad Sci USA **94**: 11786-11791