

SUPPLEMENTAL METHODS

RT-PCR

A total of 10-15 μg RNA isolated by RNeasy Mini kit (Qiagen) was treated with 2 μl of Turbo DNase I (Ambion) for 1 hour at 37°C. A second digestion was followed by adding additional 2 μl of Turbo DNase I. RNA was then cleaned up according to RNeasy Mini Protocol for RNA Cleanup (Qiagen). Purified RNA was treated with another 2 μl of Turbo DNase I to remove residual genomic DNA. After final digestion, RNA was cleaned up with RNeasy kit (Qiagen) and dissolved in nuclease free water. For RT-PCR analysis, first strand cDNA synthesis was performed using 100 ng of random hexamer, 1 μg of DNase I treated RNA template and 1 μl of Superscript III (Invitrogen) reverse transcriptase according to the manufacturer's instruction. As a negative control (-RT), the same reaction was performed without SuperScript III. To detect *act1⁺* transcript and TUKs, 20 and 33 cycles of PCR after RT-step were performed respectively, using 1/20th volume of cDNA products.

Northern analysis

Total RNA was isolated by RNeasy Mini or Midi kit (Qiagen). RNA was then precipitated with 1/10th volume of 3M NaOAc (pH 5.5) and 2.5 volume of 100% ethanol. Poly(A) containing RNA was purified from 500 μg of total RNA by affinity purification with biotinylated oligo-dT using PolyATtract mRNA Isolation Systems (Promega) following the manufacturer's protocol. 12-15 μg of total RNA or 1-1.5 μg of poly(A) RNA was run on an 1% or 1.5% agarose gel, blotted on nylon membrane (Hybond N, Amersham), UV-crosslinked and hybridized with strand-specific RNA probes. To make RNA probes, DNA fragment specific for central domain or *act1⁺* was amplified by PCR and gel-purified. Then, T7 promoter was equipped at the end of DNA fragment through another round of PCR amplification using a primer containing T7 promoter sequence. Primer sequences are described in Supplemental Table 1C. The T7 promoter containing PCR products were transcribed into [α 32P]-UTP labelled RNA probes using Strip-EZ RNA kit (Ambion). The probes were hybridized to the membranes overnight at 68°C in a rotating oven and washed with a buffer containing 2XSSC and 0.1% SDS for 30 min at 68°C twice followed by more stringent washes with a buffer containing 0.5XSSC and 0.1%SDS for 15 min at 68°C twice. To detect TUKs, the blots were analyzed after 1 day of exposure to phosphor screens. To detect *act1⁺* control mRNA, the blots were stripped according to Strip-EZ RNA Protocol (Ambion), rehybridized with *act1⁺* RNA probe and exposed to phosphor screens for 2 hours before analysis.

5'-RACE-PCR

5'-RACE-PCR was performed with 0.5 µg of oligo dT-selected RNA isolated from wild-type or *cnp1-1* grown at restrictive temperature using FirstChoice RLM-RACE kit (Ambion) according to the manufacturer's instructions. Briefly, RNA was treated with CIP to remove 5' phosphate from non 5' capped RNA species. Followed by removal of CIP, tobacco acid pyrophosphatase (TAP) was treated to digest 5' cap. After these procedures, 5' phosphate of RNA which is essential for subsequent ligation to 5'-RACE adapter is revealed only from RNA which originally contained 5' cap, thereby enabling selective amplification of 5' capped RNA. -TAP control reaction was used to assess that RACE product is specific to the 5' ends of decapped RNA. To amplify reverse transcribed products, two rounds of PCR were performed; first round with 5'-RACE outer primer corresponding to RNA adapter and a *cnt1* specific outer primer, followed by a second round (nested) PCR with 5'-RACE inner primer and a nested *cnt1* specific inner primer. For *act1⁺* and *cnt1* specific primers used in 5'-RACE-PCR, see Supplemental Table 1B. The PCR products were then gel-purified, cloned into pGEM-T Easy vector (Promega) and sequenced.