Note

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Identification of a cDNA from *Arabidopsis thaliana* Encoding a Member of the Conserved SUG1 Protein Family by Complementation Screening in Fission Yeast Meiotic Mutants

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Abstract

One family of Conserved ATPase-containing Domain (CAD) proteins, the SUG1 family, is reported as a regulatory subunit of proteasome and/or a transcriptional mediator. We isolated a cDNA for the *Arabidopsis SUG1* gene (*AtSUG1*), which shares strong homology with members of the SUG1 CAD family. Overproduction of *AtSUG1* cDNA weakly restored meiotic division in a fission yeast mutant defective in the *mei2* gene, which encodes a meiotic regulator. *AtSUG1* was expressed at an almost constitutive level, independent of plant organ or developmental stage. AtSUG1 can functionally substitute for SUG1 in budding yeast.

Conserved ATPase-containing Domain (CAD) proteins (Swaffield and Purugganan, 1997), also known as AAA proteins (Confalonieri and Duguet, 1995), have a highly conserved ATPase module with 200 amino acids, and are involved in a wide variety of cellular functions in many species. Budding yeast SUG1, a CAD protein, was originally identified as a suppressor mutation of a mutant allele of the transcriptional factor GAL4 (Swaffield *et al.*, 1992). Subsequently, SUG1 was found to be a component of RNA polymerase II (Pol II) holoenzyme (Kim *et al.*, 1994), and to be capable of direct interaction with the TATA-binding protein (TBP) in yeast (Swaffield *et al.*, 1995). However, it is still unclear whether SUG1 functions as a factor responsible for transcription, because of a report that the RNA Pol II complex does not appear to include SUG1 in its active form (Rubin *et al.*, 1996; Swaffield *et al.*, 1996). On the other hand, several CAD proteins have been shown to be subunits of the 26S proteasome, which recognizes ubiquitinylated proteins for selective proteolysis. Recent reports show that SUG1 is one of the regulatory particles of budding yeast 26S proteasome (Glickman *et al.*, 1998; Rubin *et al.*, 1998).

Mammalian homologues of SUG1 have been cloned, including p45 and Trip1 from human (Akiyama *et al.*, 1995; Lee *et al.*, 1995), and mSUG1/FZA-B/m56 from mouse (Sun *et al.*, 1997; vom Baur *et al.*, 1996; Wang *et al.*, 1996). Mammalian SUG1 proteins interact with a number of nuclear receptors or transcription factors, suggesting that there might be a link between the transcription and proteasome machinery (Fraser *et al.*, 1997; Wang *et al.*, 1996). It is also possible that the SUG1 family plays multiple roles in more than one cellular function: nuclear human SUG1 sediments not only with the PA700 regulatory proteasome complex, but also in regions corresponding to higher and lower molecular weights (Fraser *et al.*, 1997). Additionally, SUG1 had a function independent of protein degradation, according to an investigation of the budding yeast *RAD23* gene, which is a target of the proteasome (Russell *et al.*, 1999).

We isolated a member of the SUG1 protein family from *Arabidopsis* by transcomplementation in fission yeast. The overproduction of this gene can weakly suppress the meiotic-defective phenotype in the *mei2* mutant. We discuss this suppression later.

The fission yeast strains used in this study were JX155 (*h90 ade6-M216 leu1 ura4-D18 mei2-*

197E) as the host for screening, JY371 (*h90 ade6-M216 leu1 mei2-33ts*) as the *mei2* temperature-sensitive strain, and JY450 (*h90 ade6-M216 leu1*) as the *mei2+* strain, used as the positive control. The budding yeast strain used was CMY763 (*Matα cim3-1 ura3-52 leu2Δ1*), used as the host for the complementation test. *Arabidopsis thaliana* (Columbia ecotype) was used as the source for extraction of RNA.

The fission yeast is a genetically tractable microbe and is a useful model organism for the study of meiotic regulation. A number of genes related to meiosis have been identified and cloned in fission yeast (Yamamoto, 1996). One of these genes, *mei2*, is thought to be a key gene for switching the mitotic cell cycle to meiosis (Watanabe *et al.*, 1988). The *mei2* gene product was shown to be required at two distinct stages of meiosis in fission yeast, before premeiotic DNA synthesis and before the first meiotic division (meiosis I) (Watanabe and Yamamoto, 1994). One *mei2* point-mutant, *mei2-197E*, is arrested mainly before premeiotic DNA synthesis; another, *mei2-33ts*, is arrested before the first meiotic division at the restriction temperature 37 ℃ (Watanabe and Yamamoto, 1994). Using *Arabidopsis* cDNA libraries that can be expressed in fission yeast, we screened for cDNA clones that suppressed the arrested meiosis in the *mei2-197E* mutant. We obtained two kinds of cDNA clones, but the suppression was very subtle. Then one of these clones, pAME7, was transformed with another *mei2* mutant, *mei2-33ts*. After growing on a sporulation plate at a semirestrictive temperature of 30 ℃, transformed cells with pAME7 could weakly recover meiosis in comparison with transformed cells with pREP3. **Table 1** shows the sporulation rate of the *mei2-33ts* mutant with pREP3 and pAME7. The *mei2-33ts* mutant transformed with pAME7 showed a slightly elevated rate of cell meiosis, but the mutant with the pREP vector showed very little.

We determined the nucleotide sequence of the 1547-bp insert in pAME7 (accession no. AB044348). The coding region of pAME7 from the putative first methionine at position 104 to the stop codon at position 1363 encodes a 419-deduced-amino-acid peptide with a calculated molecular size of about 47 kDa. A poly(A) tail with a stretch of 26 adenine residues was found. An in-frame stop codon was present upstream from the first ATG initiation codon, which indicates that this clone may include the entire open reading frame. A homology search revealed that the deduced peptide encoded by pAME7 has a strong similarity to the SUG1 protein family. **Fig. 1** shows the amino acid alignment of a protein encoded by pAME7 and SUG1 proteins isolated from budding yeast, mammals, fungi, and fission yeast. Proteins encoded by pAME7 seem to belong to the SUG1 family for 3 significant reasons. (i) The putative protein of pAME7 is about 75% identical with any SUG1 family proteins (**Fig. 1**). This is more homologous than other ATPase family proteins. (ii) The putative protein has heptad repeats of hydrophobic amino acid residues, which is conserved in SUG1 family proteins (**Fig. 1**). (iii) The polypeptide's calculated isoelectric point of 9.25 is similar to those of SUG1 family proteins-8.35 for mammalian SUG1 and 9.37 for budding yeast SUG1-and greater than those of other ATPase subunits of 26S proteasomes (Akiyama *et al.*, 1995). Therefore, the gene on pAME7 is designed *AtSUG1*. Recently, Fu *et al.* (1999) reported *RPT* (*r*egulatory *p*article *t*riple-A ATPase) gene family as components for the proteasome of *Arabidopsis*, and AtSUG1 turned out to be identical to RPT6a (Fu *et al.*, 1999).

Table 1 Sporulation efficiency of the *mei2-33ts* mutant (JY371) transformed with pAME7 at a semirestriction temperature 30 ℃. Cells were incubated on a SSA (Egel and Egel-Mitani, 1974) plate for 6 d, and sporulation frequencies were calculated for ≥500 cells inspected under a microscope. The vector used was pREP3 (Maundrell, 1993). The *nmt1* promoter in the pREP vector was used as a constitutive promotor in this study. The wild-type (JY450) was used as the *mei2+* control.

Strain	Plasmid	Sporulation rate
mei2-33ts	pREP3	0.4%
mei2-33ts	pAME7	2.0%
wild-type		61.0%

To investigate the possibility that the *Arabidopsis* genome has other SUG1 family proteins, we performed Southern blot analysis of *Arabidopsis* genomic DNA using the pAME7 insert as a probe. Under high stringency conditions, this probe, which carries an *Xba*I cutting site and no *Eco*RI or *Hin*dIII cutting site (consistent with the DNA sequence), gave 2 intense hybridization bands and 3 weak bands in the *Xba*I digest, and 1 intense band and 1 weak band in each of the *Eco*RI and *Hin*dIII digests (**Fig. 2**). These results suggest that *AtSUG1* may have a homologue in the *Arabidopsis* genome. Indeed, we found another cDNA with strong homology with the N-terminal region of *AtSUG1* in *Arabidopsis* expressed sequence tags (GenBank:

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AtSUG1 MAAVGVDSRRPETAMEETCNVKGAAAKQGEGLKQYYLQHIHELQRQLRQK  50
SUG1                  MTAAVTSSNIVLETHES.I.P.FE.K.Q.TELKI.S.  37
mSUG1               M.LDGPEQMELEEG.A.S..R....SK.E...LIVND.  38
tbp10                             MWCNNSARD.CSK.E..EIKVNE.  24
Let1                    M.EVL.TNVLQSN.NIV...T.K.QDAELAIL..  34

                *       *       *       *       *       *       *
AtSUG1 TNNLNRLEAQRNELNSRVRMLREELQLLQEPGSYVGEVVKVMGKNKVLVK 100
SUG1   .E..R.......A..DK..FIKD..R............I.IVSDK.....  87
mSUG1  SQ..RR.QA......AK..L..........Q........RA.D.K.....  88
tbp10  AQD.R..........N.....K......TN...H.A....L.........  74
Let1   .Q..R.......G..A...L....I.............I.T.........  84

AtSUG1 VHPEGKYVVDIDKSIDITKITPSTRVALRNDSYVLHLVLPSKVDPLVNLM 150
SUG1   .Q.....I..VA.D.NVKDLKA.Q..C..S...M..K..EN.A....S..  137
mSUG1  ......F...V..N...NDV..NC.........T..KI..N......S..  138
tbp10  .N....F.....PTV..A.L.....A..KHE..T..RI..N.I....S..  124
Let1   ..........SPD...KE.K.NI.........Q.IKI..N......S..  134

AtSUG1 KVEKVPDSTYDMIGGLDQQIKEIKEVIELPIKHPELFESLGIAQPKGVLL 200
SUG1   M..........V...TK............V..................I.  187
mSUG1  M.........E......K............V.......A..........  188
tbp10  ....I.......V....K.................................  174
Let1   M...I.....E.V...EK............V...........P....I..  184

AtSUG1 YGPPGTGKTLLARAVAHHTDCTFIRVSGSELVQKYIGEGSRMVRELFVMA 250
SUG1   ......................K......A.....................  237
mSUG1  ..................................F....A..........  238
tbp10  ..................................................I..  224
Let1   ......................K.............................  234

AtSUG1 REHAPSIIFMDEIDSIGSARMESGSGNGDSEVQRTMLELLNQLDGFEASN 300
SUG1   ...................T.V. ...G.....................T.K 286
mSUG1  ...................S.L.G... .....................TK 287
tbp10  ...................S.G.....G.....................STK 274
Let1   ...................S.SD.SG.S.....................TK 284

AtSUG1 KIKVLMATNRIDILDQALLRPGRIDRKIEFPNPNEESRFDILKIHSRKMN 350
SUG1   N..II.....L....P..............P.SVAA.AE..R.......  336
mSUG1  ....I..........S..............P....A.L...........  337
tbp10  N.....C........P..................GDAG.L..........  324
Let1   N...I..........P..............P.SA.A.AE..R....S..  334

AtSUG1 LMRGIDLKKIAEKMNGASGAELKAVCTEAGMFALRERRVHVTQEDFEMAV 400
SUG1   .T...N.R.V......C...DV.G.......Y......I...........  386
mSUG1  .T...N.R....L.P......V.G.......Y...................  387
tbp10  .T...N....SD...................Y.........S........  374
Let1   .T......S..............G.........................  384

AtSUG1 AKVMKKDTEKNMSLRKLWK  419
SUG1   G...N.NQ.TAI.VA....  405
mSUG1  ....Q..S.....IK....  406
tbp10  S......S.Q...IN....  393
Let1   ...LN.GDSGE...Q....  403
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Fig. 1 Alignment of the amino acid sequence of the *AtSUG1* gene product with (in order) the gene product sequences of budding yeast *SUG1* (Ghislain *et al.*, 1993), mammalian SUG1 (Akiyama *et al.*, 1995; Sun *et al.*, 1997; vom Baur *et al.*, 1996; Wang *et al.*, 1996), *Dictyostelium DdTBP10* (Shaw and Ennis, 1993), and fission yeast *let1* (Michael *et al.*, 1994). Dots indicate amino acids identical to the *AtSUG1* gene product. Asterisks indicate the hydrophobic amino acids forming the heptad repeats of a putative leucine zipper.

ATTS0342). So far, only one SUG1 family gene has been reported from each organism. However, *Arabidopsis* may have two highly similar genes.

Mammalian SUG1 homologues can rescue *sug1* mutants in budding yeast (Lee *et al.*, 1995; Sun *et al.*, 1997; vom Baur *et al.*, 1996; Wang *et al.*, 1996). To determine whether AtSUG1 is a functional homologue of budding yeast SUG1, we transformed a budding yeast strain carrying the thermosensitive *sug1/cim3-1* mutation (Ghislain *et al.*, 1993) with a

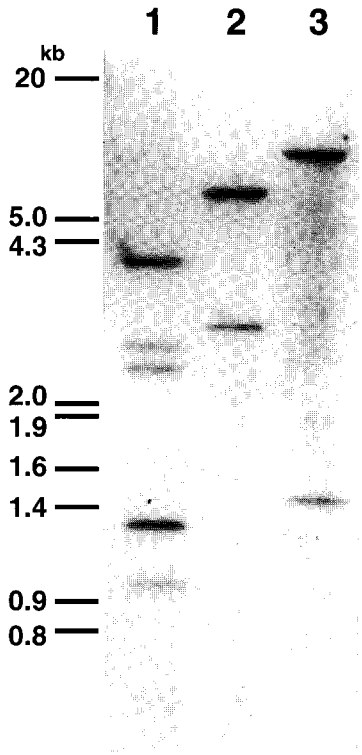

Fig. 2 Southern blot analysis of *Arabidopsis* genomic DNA. The DNA was completely digested by restriction endonucleases *Xba*I (lane 1), *Eco*RI (lane 2), and *Hin*dIII (lane 3). The digests were run in an agarose gel (1%), blotted to the membrane, and probed by an insert of pAME7. Hybridization was performed in 5 × SSC containing 50% formamide, 0.02% SDS, 0.1% N-lauroylsarcosine sodium salt, and 5% Blocking Reagent (Boehringer Mannheim) at 42 ℃ for 14 h. The membrane was washed with 2 × SSC - 0.1% SDS at room temperature for 1 min 3 times and then at 55 ℃ for 30 min.

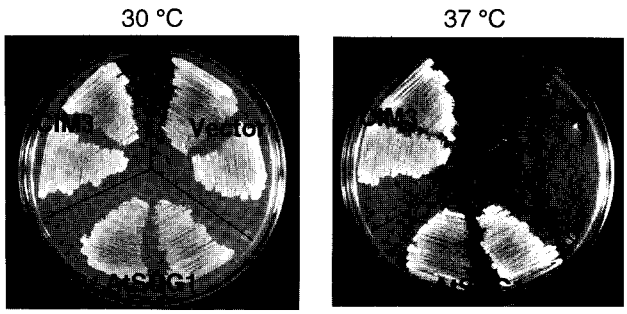

Fig. 3 Functional substitution for SUG1 in budding yeast. The budding yeast strain *cim3-1* was transformed with multicopy plasmids carrying *AtSUG1*, *CIM3*, or the vector alone, and incubated at 30 ℃ (left) or 37 ℃ (right) for 3 d. *AtSUG1* cDNA was expressed from the budding yeast expression vector pNV11, provided by Dr. C. Mann. *CIM3* was expressed in plasmid pFL44D as a positive control (Ghislain *et al.*, 1993). The vector YEp352 was used as a negative control.

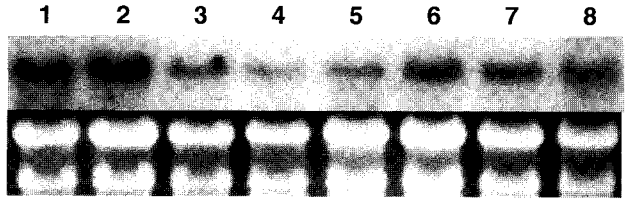

Fig. 4 Expression of *AtSUG1* in *Arabidopsis* plants. RNA was extracted from flower buds (lane 1), flowers (lane 2), stems (lane 3), and leaves (lane 4) of mature plants. RNA from whole plants was extracted 10 d (lane 5), 20 d (lane 6), 30 d (lane 7), and 40 d (lane 8) after sowing. Approximately equal loadings of RNA (about 5 μg) were verified by ethidium bromide staining of ribosomal RNA, as shown at the bottom. A 1.55-kb cDNA fragment of pAME7 was used as the probe.

vector containing the coding region for *AtSUG1* cDNA. The transformants with *AtSUG1* grew at a non-permissive temperature as well as transformants with *CIM3* (positive control) (**Fig. 3**). This indicates that AtSUG1 can perform the essential functions of SUG1 in budding yeast.

Some studies report the transcriptional expression of SUG1 family genes. *XSUG1* was shown to be exclusively expressed in *Xenopus* ovary (Nacken, 1997). 18-56 protein from moth was isolated as one of several genes that are upregulated during the programmed cell death of muscle (Sun *et al.*, 1996). Several classes of mouse neurons displayed elevated levels of m56 mRNA (Wang *et al.*, 1996). DdTBP10 from *Dictyostelium* was expressed most in vegetatively growing cells (Shaw and Ennis, 1993). Pros45 from *Drosophila* was expressed predominantly in the central nervous system (Cheng *et al.*, 1998). Thus, there is no consensus yet in the expression pattern of SUG1 family genes. We studied the organ-specific or developmental-stage-specific expression of *AtSUG1* gene transcripts by RNA blot analysis. Total RNA was extracted from flower buds, flowers, stems, and leaves of adult plants, and from whole plants at 10, 20, 30, and 40 d after sowing. Northern blot analysis revealed a single 1.6-kb RNA band in each lane, slightly stronger in lanes from flower buds and flowers than from stems and leaves. The levels at the different developmental stages were almost constitutive (**Fig. 4**). This indicates that the *AtSUG1* product might not be a peculiar factor in some organs but a basic factor in plant cells.

AtSUG1 overproduction weakly restored meiotic division in a fission yeast mutant, *mei2-33ts*, which had been arrested before the first meiotic division. How is the suppression explained? Interestingly, it has been suggested that the proteasomes are con-

cerned with chromosome separation in mitosis (Funabiki *et al.*, 1996). The *cim3* mutant in budding yeast, a mutant allele of *sug1*, was arrested in G2/metaphase at the restriction temperature (Ghislain *et al.*, 1993). This suggests that CIM3/SUG1 is required for anaphase chromosome separation. The *mei2-33ts* mutant seems to be unable to segregate the chromosomes at the restriction temperature after premeiotic DNA synthesis (Watanabe and Yamamoto, 1994). This result may show that *mei2-33ts* has a defect in proteasomal activity that results in arrest before the first meiotic division, and that *AtSUG1* overproduction could have some effect on its activity. Alternatively, the suppression may be related to transcription. The SUG1 protein family may interact with some transcriptional component (Kim *et al.*, 1994; Makino *et al.*, 1999; Swaffield *et al.*, 1995; Weeda *et al.*, 1997). *AtSUG1* overproduction might perturb the transcriptional system, resulting in the upregulation of some yeast genes that can complement the *mei2-33ts* mutant phenotype. A SUG1 family gene, *let1*, was isolated from fission yeast (Michael *et al.*, 1994). It was shown to be essential for fission yeast viability, but its function remains unclear. It is attractive to examine the relationship between Mei2 and Let1. In any case, more knowledge of the functions of *mei2* could clarify the reason for the suppression.

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