Arabidopsis cDNA Clones Isolated by Transcomplementation of the Fission Yeast cAMP Phosphodiesterase Mutant

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We isolated three kinds of Arabidopsis thaliana cDNA clones that could rescue the mating-defective phenotype of the pde1 mutant of fission yeast Schizosaccharomyces pombe, which lacked cAMP phosphodiesterase. One of them, named APS1, encoded a protein similar to Rat Arf1p GTPase-activating protein (Arf1p GAP), which has a Cys2/Cys2-type GATA-1-like zinc-finger motif, suggesting that APS1 is a novel member of this class of zinc-finger protein gene family in Arabidopsis. Disruption of the zinc-finger motif in the gene product APS1, however, did not abolish its ability to suppress pde1.

Cyclic AMP (cAMP) plays an important signaling role in initiation of sexual development in fission yeast. cAMP is synthesized by adenylate cyclase encoded by the cya1 gene, and hydrolyzed by cAMP phosphodiesterase encoded by the pde1/cgs2 gene. When the intracellular cAMP level is high, protein kinase A (PKA) is activated by the binding of cAMP to the regulatory subunit, which in turn represses transcription of the ste11 gene encoding a key transcription factor for mating, meiosis and sporulation. A decrease in the cAMP level under poor nutritional conditions triggers ste11 transcription and induces sexual development. Although cAMP is predicted to serve as a second messenger in higher plants, especially its possible relation to the regulation of sexual development, we previously set out to isolate Arabidopsis cDNAs that could suppress mating-deficiency of the pde1 end. The gene represented by APS1 was not preceded by an in-frame stop codon, we performed 5’-RACE to obtain the additional sequence at the 5’ end. We isolated several 5’-RACE clones and sequenced them. The results showed that an in-frame stop codon (TGA) was present 33 bp upstream of the first ATG, suggesting that this ATG codon was indeed the start site for translation. The total cDNA containing the complete coding region was 1.9 kb in length, and the deduced gene product had 456 amino acid residues.

The predicted gene product of APS1 showed a high similarity to Saccharomyces cerevisiae proteins.
Arabidopsis cDNA Clones Suppressing S. pombe pde1

Figure 1. Comparison of the N-terminal sequences of APS1 and similar zinc-finger proteins. See the text for explanation of these proteins. Identical amino acid residues are shown in white against black. Conserved cysteine residues in the zinc-finger motif are marked with asterisks.

Figure 2. Assignment of the region of APS1 important for the suppression of S. pombe pde1 mutant. Polypeptides encoded by a series of truncated cDNAs, generated according to a standard method, are schematically shown. The shaded box in APS1 indicates a zinc-finger motif. aps(38-456) lacks the N-terminal half of the zinc-finger. Each DNA fragment was connected to the high-copy expression vector pREP3 carrying the nmt1 promoter and was introduced into JZ666. The ability of each construct to recover mating proficiency in JZ666 is shown in (+) and (−) on the right.

designated Gcs1p, Glo3p, and SpS18p in the N-terminal 107 amino acid residues (44%, 34%, and 31% identity, respectively) (Fig. 1). It was even more similar in the same region to Rat Arf1p GAP (53% identity and 67% similarity) (Fig. 1). The overall identity of APS1 to Arf1p GAP was 26%. All these proteins carry a conserved CXXCX6CXXC motif (where X is any amino acid), which apparently represents a zinc-finger structure, in the N-terminal region. Arf1p is a GTP-binding protein related to the mechanisms underlying the cycling of the coat protein in Golgi complexes. The zinc-finger motif in these proteins (CXXCX6CXXC) is similar to the DNA binding domain of GATA-1 transcription factor (CXXCX6CXXC). In Arabidopsis, CONSTANS, COL2, and STO have been reported to have two GATA-1-like zinc-finger domains. The CONSTANS gene promotes flowering. APS1 apparently is a novel member of the gene family encoding GATA-1-like zinc-finger proteins in Arabidopsis.

To determine which part of APS1 is essential for suppression of the pde1 mutation, we tested a series of deletion constructs for their ability to suppress the pde1 mutant (Fig. 2). pAPS4 carried a truncated form of from stationary phase to G1 phase in the cell division cycle, and the zinc-finger structure is important for the activity of these gene products. The zinc-finger motif in these proteins (CXXCX6CXXC) is similar to the DNA binding domain of GATA-1 transcription factor (CXXCX6CXXC). In Arabidopsis, CONSTANS, COL2, and STO have been reported to have two GATA-1-like zinc-finger domains. The CONSTANS gene promotes flowering. APS1 apparently is a novel member of the gene family encoding GATA-1-like zinc-finger proteins in Arabidopsis.

To determine which part of APS1 is essential for suppression of the pde1 mutation, we tested a series of deletion constructs for their ability to suppress the pde1 mutant (Fig. 2). pAPS4 carried a truncated form of
APS1, missing half of the zinc-finger motif (aps 38–456 in Fig. 2). It could suppress pde1 as effectively as pAPS1. Deletion of residues 1–167 (aps 168–456), which removed the zinc-finger motif completely, weakened the suppression significantly but not completely. Additional deletion of residues 386–456 (aps 135–385) resulted in the complete loss of suppression, suggesting that the C-terminal region is important for suppression. Residues 1–134 (aps 1–134), which included the zinc-finger domain, did not suppress pde1. These results imply that the C-terminal region may be responsible for suppression of the pde1 mutation and that the zinc-finger region may enhance suppression. However, no known conserved motif was found in residues 386–456.

The S. pombe ste11 gene encodes a transcription factor of the HMG-family. Ste11p activates transcription of a number of genes required for sexual development, and expression of ste11 is repressed in the pde1 mutant due to elevated intracellular cAMP levels. Because APS1 is similar to the transcription factor GATA-1, we investigated whether pAPS1 could complement the S. pombe ste11 mutant. The result was negative (data not shown), consistent with the finding that the zinc-finger motif is not necessary for the suppression of pde1, indicating that APS1 does not substitute Ste11p. Thus, APS1 may promote expression of ste11 in the presence of a high level of cAMP. Alternatively, given its similarity to Arf1p GAP, APS1 may possibly affect more mechanical aspects of mating and sporulation. It will be intriguing to see whether APS1 or other C2/C2-type zinc-finger proteins have any relation with the cAMP cascade in Arabidopsis.

We examined transcription profiles of the isolated cDNAs by Northern blot analysis, using the inserts as probes. To investigate tissue-specificity or developmental stage-specificity of expression of each clone, we examined the respective transcription level in extracted RNA from flower bud, flower, stem, leaf, and from 10, 20, 30, and 40 day-old whole plants. The transcription level of APS1 (Fig. 3) and that of APS2 (data not shown) were fairly constant in any part and at any developmental stage of the plant. The transcription level of APS3 was apparently low in the stem and in the 20 day-old plants (data not shown), which was consistent with our observation that stems of Arabidopsis grow actively around 20 days after seeding.

APS1 was recently recorded as At2g37550 by the Arabidopsis genome project.20 Our genomic Southern analysis has indicated that Arabidopsis carries no other gene that strongly cross-hybridizes with APS1 (data not shown). However, computer analysis predicts that the product of a putative Arabidopsis gene “F5K20.10”20 is nearly 80% identical with APS1 over the entire length. Whether the two proteins share the same function remains to be seen.

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