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The *Schizosaccharomyces pombe pde1/cgs2* Gene Encodes a Cyclic AMP Phosphodiesterase

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We previously reported the identification of eight *Sz. pombe* cDNA clones that are capable of suppressing the heat-shock sensitive phenotype associated with deletion of *IRA1* in *S. cerevisiae*. We report that one of these cDNA clones, pPSI5, encodes a protein, Pde1, that is 24% identical to the *S. cerevisiae* low-affinity cAMP phosphodiesterase. The *pde1/cgs2* gene encoding this protein has been previously identified, and studies have shown that deletion of this gene results in elevated levels of intracellular cAMP and inhibition of meiosis. To demonstrate that Pde1 is a cAMP phosphodiesterase we expressed it in an *S. cerevisiae* strain which lacks the genomic cAMP phosphodiesterase genes. Extracts from such cells that express the *Sz. pombe* Pde1 exhibit high levels of cAMP phosphodiesterase activity.

cAMP acts as an important second messenger in many, if not all, eucaryotes. Yet cAMP signaling pathways appear to control different responses in different organisms. In the fission yeast *Sz. pombe* cAMP appears to regulate mating and meiosis. Agents which stimulate the cAMP pathway (1, 3, 24), overexpression of adenylyl cyclase (10), or a mutation in the regulatory subunit of the cAMP dependent protein kinase gene, *cgs1*, (8) inhibits conjugation and sporulation, while deletion of adenylyl cyclase leads to hyper-sexual development (10, 12). In contrast, the cAMP pathway of the budding yeast *S. cerevisiae* does not appear to be involved in sexual development. Attenuation of this pathway strongly inhibits cell growth (9, 13, 22). Activation of the cAMP pathway in *S. cerevisiae* leads to several phenotypes, including failure to arrest in the G1 phase of the cell cycle and failure to accumulate storage carbohydrates upon nutrient starvation, and sensitivity to heat-shock treatment or nitrogen starvation (4, 9).

Deletion of the *IRA1* gene in *S. cerevisiae* leads to activation of the cAMP pathway. Ira1 is a negative regulator of Ras1 and Ras2 (19, 20), which are positive regulators of adenylyl cyclase (2, 23). We have previously identified eight *Sz. pombe* cDNAs that are capable of suppressing the heat-shock sensitive phenotype due to deletion of *IRA1* in *S. cerevisiae* (14). Three of the

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cDNA clones we isolated could also suppress deletion of *PDE1* and *PDE2*, the two *S. cerevisiae* cAMP phosphodiesterase genes. We suspect that such clones encode proteins that act downstream from adenylyl cyclase, and thus may be conserved components of the cAMP pathways of these distantly related yeasts. The aim of this study was to further characterize one of these cDNA clones, pPSI5.

MATERIALS AND METHODS

Yeast strains and genetic analysis: The *S. cerevisiae* strain 10DAB (*Matα his3 leu2 ura3 ade8 pde1::ADE8 pde2::URA3 ras1::HIS3*) has been previously described (5). Yeast growth, transformation and other methods used have been previously described (17).

Plasmids and DNA analysis: pPSI5 contains a *Sz. pombe* cDNA cloned in the yeast expression vector pADANS, as previously described (14). DNA sequences were determined in both orientations using the Taq DyeDeoxy Terminator Cycle Sequencing Kit (Applied BioSystems).

cAMP phosphodiesterase assay: cAMP phosphodiesterase activities were measured in yeast cell extracts by a modification of a previously described method (11, 21). A 500 ml yeast culture was grown in synthetic (SC) media to late log phase (OD_{600} = 1.0). Cells were washed in Buffer X (50 mM Tris, pH 8.0, 1 mM EDTA, 1 mM β-mercaptoethanol, 0.5 mM PMSF), resuspended in 40 mls of Buffer X and lysed in a French Press (20,000 lb/in^2). Lysed cell extracts were spun at 1600g for 10 minutes, the supernatants were respun at 18,000g for 20 minutes, and cAMP phosphodiesterase activities in the resulting supernatants were measured, as follows. Protein concentrations were determined using a kit (Bio-Rad, Inc.). 200 µl reactions mixtures containing 0.1 M Tris, pH 8.0, 5 mM $MgCl_2$, 50 µM cAMP, 10^5 cpm ^{3}H-cAMP (New England Nuclear), and 0-200 µg yeast extract protein were incubated at 30°C for 20 minutes, followed by incubation at 98°C for 2 minutes. 200 µl 100 mM Tris, pH 8.0, 0.05 mM $MgCl_2$, and 200 µg *C. adamanteus* venom was added to each reaction mixture and incubated at 30°C for 20 minutes, followed by incubation at 98°C for 2 minutes. 1.2 ml of 30% Dowex 2-X8, 34% ethanol slurry was added to each reaction, followed by rotation at 22°C for 15 minutes. Samples were spun in a microfuge, and the ^{3}H in the supernatants was counted.

RESULTS

The DNA sequence of pPSI5 revealed that it encodes a protein, Pde1, that is homologous to the *S. cerevisiae* low-affinity cAMP phosphodiesterase. The *Sz. pombe pde1/cgs2* gene encoding Pde1 has been previously identified (8, 15). The cDNA contained in pPSI5 lacks the 5' untranslated region and first codon, but it is fused to the first 10 codons of the ADH1 coding sequence contained in the yeast expression vector pADANS.

Comparison of the *Sz. pombe* and *S. cerevisiae* Pde1 proteins revealed that they are 24% identical (15). Studies have shown that deletion of *pde1/cgs2* results in elevated levels of cAMP, and that mutation of this gene inhibits meiosis (8, 15). Together, these observations suggest that *pde1/cgs2* encodes a cAMP phosphodiesterase, but they do not rule out other possible mechanisms by which Pde1 could affect cAMP levels. To determine if Pde1 is a cAMP phosphodiesterase, we measured cAMP phosphodiesterase activities in extracts from the *S. cerevisiae* strain 10DAB transformed with either pPSI5 or the vector pADANS (Figure 1). In the strain 10DAB the *S. cerevisiae* cAMP phosphodiesterase genes, *PDE1* and *PDE2*, have been replaced with selectable markers. No measurable cAMP phosphodiesterase activity was detected

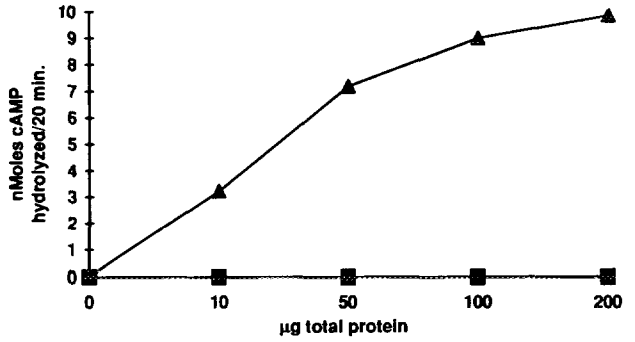

FIGURE 1. This graph shows cAMP phosphodiesterase activities measured in cell extracts from the *S. cerevisiae* strain 10DAB (*pde1- pde2-*) containing either the yeast expression vector pADANS (■) or the plasmid pPSI5 (▲) which encodes the *Sz. pombe* Pde1. Each point represents the average of three independent measurements, performed as described in Materials and Methods.

in 10DAB cells harboring pADANS. In contrast, 10DAB cells harboring pPSI5 contained high levels of cAMP phosphodiesterase activity, demonstrating that pPSI5 does encode a *Sz. pombe* cAMP phosphodiesterase.

DISCUSSION

cAMP phosphodiesterases play an important role in the regulation of intracellular cAMP levels. *S. cerevisiae* contains two distinct cAMP phosphodiesterases: Pde1, a low-affinity enzyme, and Pde2, a high-affinity enzyme (16, 18). These proteins do not share significant sequence homology with each other. The presence of two distinct cAMP phosphodiesterases suggests the possibility that they belong to different regulatory pathways. With the exception of the *D. discodeum* Pde, the known cAMP phosphodiesterases of other organisms are related to Pde2. These include the *D. melanogaster* dunce protein (7) and related mammalian Pdes (6).

We have isolated a *Sz. pombe* cDNA encoding a Pde1 related cAMP phosphodiesterase by its ability to suppress activation of the Ras/cAMP pathway in *S. cerevisiae*. The homology between this protein and the *S. cerevisiae* and *D. discodeum* cAMP phosphodiesterases is significant, but not strong enough to assume that these proteins are functionally conserved. While the genetic data and cAMP measurements suggest a role for *Sz. pombe* Pde1 in regulating cAMP levels, they do not distinguish between possible mechanisms. Our results provide conclusive biochemical evidence that the *Sz. pombe* Pde1 protein is a cAMP phosphodiesterase. It is not known whether *Sz. pombe* also contains a Pde2 related enzyme, but further investigation may reveal the presence of such a homolog.

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