EXPRESSION PROFILE AND KINASE ACTIVITY OF A CDC2-RELATED PROTEIN KINASE FROM THE AQUATIC FUNGUS BLASTOCLADIELLA EMERSONII

Ribichich, K. F. and Gomes, S. L.

Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, BRAZIL

Cyclin-dependent kinases are key enzymes responsible for the control of cell cycle progression in eukaryotes. In fungi, one Cdk (Cdk1) has been described as directly involved in the control of cell cycle transitions, differently from animals and plants where at least two Cdks and members from two distinct groups have been indicated, respectively. Although several Cdks have been characterized in different fungi, none have been identified in chytrids. We report the cloning of a full-length cDNA encoding a putative Cdk from the chytrid B. emersonii (BeCrk1) and its characterization by nucleotide sequence analysis, mRNA levels, kinase activity and kinase inhibition along the fungal life cycle. The protein sequence presented the conserved motifs of Cdks, but the characteristic signature PSTAIRE region in its cyclin-binding domain shows two substitutions. Crk1 mRNA showed an accumulation during both germination and sporulation phases of the life cycle. Kinase associated activity was investigated in immunoprecipitates obtained with a specific polyclonal antiserum and in co-precipitates with p13^{Suc1}, a high-affinity Cdk regulatory subunit that associates with active enzyme complex. The histone H1 phosphorylation patterns with both precipitates showed kinase activity throughout the life cycle with a reduction at the end of sporulation, which accompanied the mRNA profile. Moreover, both activities were inhibited with purvalanol A, a selective and potent Cdk inhibitor.