THE ORF NCU08772 ENCODES A PUTATIVE MULTIFUNCTIONAL CYCLIN INVOLVED IN GERMINATION, CELL CYCLE REGULATION, CALCIUM STRESS, AND GLYCOGEN METABOLISM IN NEUROSPORA CRASSA

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Neurospora crassa has been widely used as a model organism for fundamental aspects of eukaryotic biology. In a Blast search, we found the ORF NCU08772 product as the Saccharomyces cerevisiae Pcl10 cyclin homologue (32% identity). In yeast, the Pcl10p, together with the Pho85 cyclin-dependent protein kinase, phosphorylates glycogen synthase, the regulatory enzyme in glycogen synthesis. Phosphorylation results in enzyme inactivation and, therefore, decreased glycogen accumulation. The N. crassa \(\Delta\)pcl-10 strain showed a delay in germination and a shift in the timing of cell division when compared to the wild-type strain suggesting that this cyclin regulates fungal development and cell division. The knockout strain accumulated higher levels of glycogen than the wild-type strain during growth. The glycogen synthase (GSN) phosphorylation, and its activation state, was monitored by the \(-G6P/+G6P\) activity ratio, higher levels correlating with greater activity. The mutant strain showed lower phosphorylation, and then higher activity during growth, a result that explains the hyper-accumulation of glycogen. The GSN phosphorylation profile was analyzed by 2D-PAGE followed by Western blot using anti-GSN antibody. GSN showed less phosphorylated isoforms in the \(\Delta\)pcl-10 strain indicating a role of the cyclin in GSN phosphorylation. The N. crassa recombinant proteins PHO85, PCL-10 and GSN were produced in E. coli and used in in vitro phosphorylation assays. GSN was in vitro phosphorylated by the complex PHO85/PCL-10 and the putative phosphorylation site (Ser636) was determined. The \(\Delta\)pcl-10 strain was more resistant to calcium stress when compared to the wild-type strain. In addition, qPCR analysis showed the involvement of the cyclin in the regulation of the expression of genes related to calcium metabolism under calcium stress. We conclude that the cyclin PCL-10 in N. crassa is a multifunctional cyclin that regulates essential cellular processes. Supported by FAPESP, CNPq