Neurospora crassa has been widely used as a model organism for fundamental aspects of eukaryote organisms biology. Our laboratory has been extensively studying the biochemical and molecular mechanisms involved in glycogen metabolism regulation in this fungus. In Saccharomyces cerevisiae, PHO85 encodes the cyclin-dependent protein kinase (Cdk) catalytic subunit with multiple regulatory roles depending on its association with different cyclin partners (Pcls). Upon binding to Pcl10p, Pho85p directly phosphorylates the predominant form of glycogen synthase (Gsy2p) at a physiologically relevant site to an extent that causes the enzyme inactivation. Pho85p alone displayed no detectable activity towards Gsy2p, but addition of Pcl10p generated significant Gsy2p kinase activity. Search for Pho85p and Pcl10p orthologues in the N. crassa database retrieved a putative Pcl10-like protein encoded by the ORF NCU08772, and a Pho85-like protein encoded by the ORF NCU07580. Blast analysis and sequence alignment among proteins from different microorganisms indicate they are the putative Pho85p and Pcl10p orthologues. Both ORF NCU08772 and NCU07580 sequences were amplified by RT-PCR and the recombinant proteins were produced in E. coli as His-tag fusion protein. The proteins were produced in a soluble form, purified by affinity chromatography and the N. crassa complex Pcl10-like/Pho85-like will be further used to perform in vitro phosphorylation assays using N. crassa recombinant glycogen synthase produced in E. coli.

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