THE ROLE OF 5' NUCLEOTIDASE IN THE REGULATION OF MORPHOGENESIS IN *Dictyostelium discoideum*

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(ABSTRACT)

5' Nucleotidase (*5NU*) in *Dictyostelium discoideum* is an enzyme that shows high substrate specificity to 5'AMP. The enzyme has received considerable attention in the past because of the critical role played by cyclic AMP in cell differentiation in this organism. Degradation of cAMP by cAMP phosphodiesterase (PDE) produces 5'AMP, the substrate of 5NU. *Dictyostelium* switches its genetic program from growth to cellular differentiation when nutrients become limited. During the time course of development, the activity of 5NU is high and becomes restricted to a narrow band of cells that form the interface between the prestalk/prespore zones. Understanding how this gene is regulated will provide knowledge underlying the process of cell differentiation. In order to understand the functional significance of the *5NU*, I first purified of the 5NU protein using an artificial substrate *p*-nitrophenol phosphate (*p*NPP). An activity stain on non-denaturing gels with Nitro Blue Tetrazolium (NBT) and 5-Bromo-4-Chloro-3-Indolyl Phosphate (BCIP) as the substrate was also used. A polypeptide of approximately 90 kDa was associated with 5NU enzyme activity after gel filtration chromatography and denaturing gel electrophoreses. Protein sequence of this peptide was obtained from Mass Spectrometry and Edmund Degradation. Various databanks were searched for similar sequences, but no matches with high identity were obtained. However, a search of the sequences of an ongoing cDNA project at the University of Tsukuba in Japan revealed a clone that corresponded to the peptide sequence of *5NU*. In addition, a clone was found that corresponded to the classical "alkaline phosphatase" found in several organisms. Analysis of the expression of the *5NU* and *AP* during *Dictyostelium* development by Northern blotting determined that the *5NU* is developmentally regulated while the *AP* is expressed at all stages of the life cycle. Southern blot analysis showed a single form of the gene for both *5NU* and *AP*. Targeted gene disruption and knockout mutagenesis using the *5NU* sequences flanking a blasticidin-resistant cassette was attempted. Analysis of the transformants showed the *5NU* gene was not disrupted, and that the blasticidin-resistant cassette was randomly inserted into the genome