FOOTPRINT ANALYSIS OF THE TRANSCRIPTIONAL CONTROL OF *GLYCOGEN PHOSPHORYLASE 2* IN *DICTYOSTELIUM DISCOIDEUM*

by

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Thesis submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

Master of Science

in

Biology

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December,1997

Blacksburg, Virginia

Key words: DNase I footprint analysis, transcription, gene expression, AT-rich, looping, large footprints, replication protein A, gene expression, cell differentiation, *Dictyostelium discoideum*

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(ABSTRACT)

Glycogen phosphorylase 2 (gp-2) is a key enzyme during the development of *Dictyostelium discoideum*. The gp-2 enzyme breaks down glycogen into glucose monomers that are subsequently used to synthesize the terminal end products of cellular differentiation. This gene is an ideal candidate for studying the process of selective gene expression because its product figures so prominently in the development of this organism, implying a dependable control mechanism responsible for its developmentally regulated expression. I present in this thesis the identification of several putative *cis*-acting elements of *gp-2* as revealed through footprint analysis. Due to the extreme AT-bias characteristic of *Dictyostelium* promoters, footprinting conditions required intensive optimization with respect to template, nonspecific competitor, source of protein extract and DNase I digestion. Using an endlabeled fragment containing seven repeated sequences (3 TA boxes [TAATTATA], 2 TAG boxes [TAAAAATGGT] and 2 C boxes [ACCCACT]), purified replication protein A and several developmental nuclear extracts were tested for DNA binding activity. Small footprints were observed on the TAG and C boxes of the promoter for both protein sources. However, using a more sensitive footprinting strategy involving multiple rounds of primer extension, larger footprints spanning the same promoter regions were detected. In both cases, the appearance of the footprints coincided with the documented transcriptional activity of the gene. It can be concluded from the data obtained that the TAG and C boxes are very likely *cis*-acting elements involved in the regulation of *gp-2* expression.