

## ACKNOWLEDGMENTS

I wish to extend my thanks to Dr. Rutherford for giving me the opportunity to study in his laboratory, for leading and helping me to achieve my goals to be a good scientist. He has encouraged me, prepared me for the next step from the first day I came to the lab. Not only did he tell us how to work and study, but he actually also showed us by working on the bench doing experiments with us, like us. From the system established in the lab, lab meetings, one to one discussions and team working, I feel that I have gained a lot compared to the time I came into this lab. Finally, I thank him for his understanding, patience, professionalism and sense of humor.

I am also really grateful to Dr. Reyna Favis to whom this thesis and I owe a lot. I thank her for everything she has done for me, for devoting her valuable time to teach me both laboratory skills and theoretic approaches with patience and understanding, for helping me to write this thesis. I have been impressed and motivated by her diligence in work, enthusiasm to renew and professionalism over solving difficult problems we have faced. Most importantly, she always knew when to be a teacher and when to be a friend. I am really glad to have had the chance to be her student.

I would next like to thank my committee members, Dr. Asim Esen and Dr. Ann Stevens for their valuable suggestions, help and time.

My thanks are also for other members of our laboratory, present and past: Dr. Ian McCaffery, Laura, Chanpen, Nikita, Xiao, Beth and Jodie, for their friendship, help and sharing.

I would next like to thank the Ministry of Education of the Turkish Government and its branch, Newyork Educational Attaché for providing me with the scholarship to pursue my degree in the USA, and for their help and hard work.

Finally, I would like to thank my parents, my sisters and my brother for their love, support and encouragement.

# Table of Contents

<b>1. Introduction</b> .....	1
The Life Cycle of <i>Dictyostelium discoideum</i> .....	3
Developmental Effects of Morphogenic Signals: cyclic AMP (cAMP), Differentiation Inducing Factor (DIF), adenosine and ammonia:.....	4
Glycogen metabolism.....	5
Transcriptional Regulation of <i>gp-2</i> expression.....	7
Replication Protein A (DdRPA).....	8
<b>2. Materials Methods</b>	
Cell Culture and Harvest.....	10
Nuclear extract preparation.....	10
Protein (DdRPA) extract preparation.....	11
DEAE Sephacel Batch Assay.....	11
DNA Affinity Chromatography.....	12
Gel shift assay.....	12
Footprint.....	12
NADIR (New APEX derived in vitro reaction)(Primer Extension Footprinting).12	
Template preparation.....	13
Primer preparation.....	13
In vitro reaction.....	13
Conventional Footprint Strategy (Endlabeling Footprint Technique).....	15

Template preparation.....	15
Primer preparation.....	15
Polymerase chain reaction (PCR).....	15
In vitro reaction.....	16
Marker generation.....	16

### 3. Results

Optimizing The Conditions For Footprint Assays.....	18
Footprint of Replication Protein A (DdRPA) .....	21
C Box Region Footprints.....	22
Endlabeling Footprint Technique.....	22
Primer Extension Footprinting.....	22
TAG Box Footprint.....	23
Other Footprints with DdRPA.....	23
Footprints of Nuclear Extract Proteins During Development.....	24
C Box Region Footprints.....	24
Endlabeling Footprint Technique.....	24
Primer Extension Footprinting.....	25
TAG Box Region Footprints.....	26
Endlabeling Footprint Technique.....	26
Primer Extension Footprinting.....	26
Other Footprints with Nuclear Extracts.....	27

**4. Discussion.....28**

**Literature Cited.....37**

**Curriculum Vitae.....43**

## List of Illustrations

- Figure 1.1. Asexual life cycle of *Dictyostelium discoideum*
- Figure 1.2. Glycogen metabolism during development in *Dictyostelium discoideum*
- Figure 1.3. The sequence of the *gp-2* promoter
- Figure 1.4. 5' deletion analysis of the *gp-2* promoter in *Dictyostelium discoideum*
- Figure 2.1. *gp-2* promoter with primers
- Figure 2.2. Comparison of the two footprint techniques
- Figure 3.1. Gel shift assay to test the activity of purified DdRPA from undifferentiated and differentiated cells that binds to the 3'C probe
- Figure 3.2. Test of DNase I digestion times on the PCR460660-9001100 template and its cloned counterpart using primer extension footprinting
- Figure 3.3. DNase I footprint assay to test the effect of digestion times on template with and without the addition of protein
- Figure 3.4. DNase I footprint assay to determine the effects of extract concentration
- Figure 3.5. DNase I footprint assay to compare patterns of protection generated by DdRPA from differentiated cells (O/N), ameoba and slug extracts
- Figure 3.6. DNase I footprint assay to compare patterns of protection generated by DdRPA from differentiated (O/N) and undifferentiated (6h) cells over a large region of the promoter
- Figure 3.7. DNase I footprint assay comparing protected regions over the TAG box region generated by DdRPA from differentiated (O/N) and undifferentiated (6h) cells
- Figure 3.8. DNase I footprint assay to compare extracts from DdRPA of differentiated (O/N) and undifferentiated (6h) cells, ameoba, slug (13h) and slug (17h)
- Figure 3.9. DNase I footprint assay of DdRPA from differentiated (O/N) and undifferentiated (6h) cells, ameoba, slug (13h) and slug (17h) extracts using the endlabelling footprinting technique

Figure 3.10. DNase I footprint assay of aggregation (8h), first finger (16h) and culmination (21-22h) extracts using the endlabelling footprinting technique

Figure 3.11. DNase I footprint assay to detect developmentally regulated footprints

Figure 3.12. DNase I footprint assay to detect developmentally regulated footprints

Figure 3.13. DNase I footprint assay to test the effect of increasing amounts of first finger (16h) nuclear extract

Figure 3.14. DNase I footprint assay to detect developmentally regulated regions of protection surrounding the TAG boxes

Figure 3.15. DNase I footprint assay to detect regions of protection over a large portion of the promoter

Figure 3.16. DNase I footprint assay of ameoba with high density, aggregation (8h), first finger(16h) and culmination(21-22h) extracts using the primer extension footprinting technique

Figure 4.1. A proposed model of *gp-2* expression in *Dictyostelium discoideum*

Figure 4.2. Protein-protein interactions in the model

## List of Tables

Table A. Summary of DdRPA footprints

Table B. Summary of nuclear extract footprints on C and TAG box regions