



Table of Contents

No.	Title	Page No.
Chapter 1	Introduction	1-75
1.1	Cell death	1
1.1.1	Classification based on morphology of different cell death processes	2
1.1.2	Molecular mechanisms of apoptosis and paraptosis	2
1.1.3	Selective permeabilization of organelle membranes during cell death	4
1.1.4	Links between lysosomes and mitochondria	10
1.1.5	Calpain: an addition to the cell death complexity	11
1.2	Main proteins in Paraptosis	13
1.2.1	Poly(ADP-Ribose) Polymerase (PARP)	13
1.2.2	Apoptosis Inducing Factor (AIF)	22
1.3	<i>Dictyostelium discoideum</i>	30
1.3.1	Molecular aspects of <i>D. discoideum</i> development	33
1.3.2	<i>D. discoideum</i> as a model organism	40
1.3.3	<i>D. discoideum</i> and programmed cell death	40
	References	42
Objectives		69
Chapter 2	Materials and Methods	70-85
Chapter 3	Role of Poly {ADP-Ribose} Polymerase (PARP) in UV-C induced cell death in <i>D. discoideum</i>	86-106
3.1	Introduction	86
3.2	Results	87
3.2.1	Induction of cell death in <i>D. discoideum</i> cells by UV-C irradiation and the delay induced by PARP inhibition	87
3.2.2	PARP activation and its inhibition by benzamide under UV-C stress	89

3.2.3	PARP activation leads to adenine and pyridine nucleotides depletion	90
3.2.4	MMP changes after UV-C exposure in context to PARP activation	92
3.2.5	AIF translocation is downstream to PARP activation	94
3.2.6	Monitoring the release of cytochrome c from mitochondria	95
3.2.7	UV-C induced paraptosis leads to large scale DNA fragmentation	95
3.2.8	Characterization of paraptotic vesicles	96
3.2.9	Effect of broad caspase inhibitor on UV-C induced cell death	97
3.2.10	Effect of MEK inhibition on cell death	98
3.3	Discussion	99
3.4	References	103
Chapter 4	Role of Poly {ADP-Ribose} Polymerase (PARP) during starvation induced cell death in <i>D. discoideum</i>	107-128
4.1	Introduction	107
4.2	Results	108
4.2.1	Characterisation of starvation induced cell death	108
4.2.2	Levels of ATP and NAD ⁺ during starvation	109
4.2.3	MMP changes during starvation	110
4.2.4	ROS generation during starvation	111
4.2.5	DNA damage by immunofluorescence	112
4.2.6	Effect of PARP inhibition on starvation induced cell death	113
4.2.7	PARP inhibition affects NAD ⁺ depletion	114
4.2.8	MMP changes during starvation in context to PARP	115
4.2.9	Protease involvement in starvation induced cell death and development	116
4.2.10	PARP and <i>D. discoideum</i> development	121
4.3	Discussion	122
4.4	References	126

Chapter 5	PARP in staurosporine induced cell death in <i>D. discoideum</i>	128-141
5.1	Introduction	128
5.2	Results	128
5.2.1	Induction of cell death by STS	128
5.2.2	Mitochondrial changes induced by STS	130
5.2.3	PARP acitivity and NAD levels during STS induced cell death	145
5.2.4	Effect of STS on DNA	133
5.2.5	Effect of cathepsin D inhibition on STS induced cell death	134
5.3	Discussion	137
5.4	References	139
Chapter 6	Response of <i>Dictyostelium discoideum</i> to UV-C and involvement of PARP	142-154
6.1	Introduction	142
6.2	Results	142
6.2.1	Cell death and PARP activation induced by UV-C radiation	142
6.2.2	<i>D. discoideum</i> growth under UV-C stress	144
6.2.3	<i>D. discoideum</i> development under UV-C stress	145
6.2.4	Effect of UV-C and PARP inhibition second generation of <i>D. discoideum</i> cells	148
6.3	Discussion	150
6.4	References	153
Chapter 7	cAMP mediated chemotaxis during UV-C induced <i>D. discoideum</i> developmental changes	155-168
7.1	Introduction	155
7.2	Results	156
7.2.1	Effect of UV-C irradiation on number and size of developing bodies of <i>D. discoideum</i>	156

7.2.2	Synergistic development by mixing UV-C treated GFP tagged <i>D. discoideum</i> cells with healthy cells	156
7.2.3	Effect of cAMP on UV-C induced <i>D. discoideum</i> development	158
7.2.4	Expression kinetics of different genes after UV-C exposure	160
7.2.5	UV-C induced developmental changes restored by iNOS inhibitor	160
7.3	Discussion	162
7.4	References	166
Chapter 8	Down-regulation of AIF in <i>D. discoideum</i>	169-184
8.1	Introduction	169
8.1.1	Strategy for targeted down-regulation of <i>aif</i>	171
8.2	Results	173
8.2.1	Cloning of AIF antisense and downregulation of AIF	173
8.2.2	Effect of AIF dR on development	175
8.3	Discussion	175
8.4	References	179
	Concluding remarks	184
	Publications and Presentations	