Fig. No.	Title	Page No.	
Ch 1: Introduction			
1.1	PARP-1 protein structure containing three domains	3	
1.2	Structural organization of human PARP-1 (hPARP-1)	5	
1.3	Mechanism of PARP-1 Activation	7	
1.4	Multifunctional roles of PARP-1	9	
1.5	Multiple modes of transcriptional regulation by PARP-1	12	
1.6	PARP-1 modulates stress responses at multiple levels	13	
1.7	Poly(ADP-ribose) polymerase 1 (PARP-1) mediated cell death	15	
1.8	PARP-1 and cancer therapy	18	
1.9	The life cycle of Dictyostelium discoideum	21	
1.10	Arrangement of cell types in <i>Dictyostelium</i> slug and culminants	23	
1.11	Dictyostelium discoideum cAMP signalling system	28	
Ch 2 : Materials and Method			
2.1	Validation of ADPRT1A knockout strain.	48	
Ch 3: Phylogenetic analysis of Poly (ADP-ribose) polymerase-1 in D. discoideum			
3.1	Phylogenetic analysis of PARP isoforms in <i>D. discoideum</i> and	60	
	human PARP by maximum likelihood method		
3.2	Pictorial representation of domain organization of ADPRT1A,	61	
	ADPRT1B and ADPRT2 by Pfam and Prosite.		
3.3	(A) Schematic representation of domain organization and (B) the	62	
	positions in ADPRT1A by Pfam, Prosite and InterPro software.		
3.4	Multiple alignment of protein sequences of PARP-1 from human	64	
	and its orthologs from <i>M. musculus</i> , <i>D. melanogaster</i> , <i>C. elegans</i>		
	and <i>D. discoideum</i> (ADPRT1A).		
3.5	Unrooted phylogenetic tree of PARP-1.	65	
Ch 4 : Over expression of ADPRT1A and its effects on <i>D. discoideum</i> growth and			
multicellularity			
4.1	PCR amplification of full length ADPRT1A	73	
4.2	Colony screening by RE digestion	74	
4.3	Clone confirmation of ADPRT1A-EYFP by RE digestion	75	
4.4	Clone confirmation of untagged ADPRT1A construct by RE	75	
	digestion		
4.5	Functional characterization of ADPRT1A overexpression	76	
4.6	Localization of ADPRT1A-EYFP in D. discoideum cells and	78	
	transcript analysis of ADPRT isoforms in A OE.		
4.7	Percent change of NAD ⁺ concentration in control and ADPRT1A-	79	

List of figures

	EYFP OE using Bernofsky's Enzymatic recycling method.	
4.8	Growth analysis of Control, ADPRT1A-EYFP OE (AE OE) and	80
	EYFP vector control cells.	
4.9	Analysis of cells cycle by FACS using Propidium Iodide.	81
4.10	ROS and PARP inhibition in <i>ADPRT1A</i> OE (A OE).	82
4.11	Fluorimetric analysis of ROS levels using DCFDA dye in control	83
	and ADPRTIA OE (A OE) cells.	
4.12	DNA damage was observed in control and A OE 5 min post	84
	0.03mM cumene H ₂ O ₂ stress by immunofluorescence using antibody	
	against pH2AX.	
4.13	PARP activation post 0.03mM cumene H ₂ O ₂ treatment using anti-	85
	PAR antibodies in control, PARP inhibitor control and A OE cells	
	by confocal microscopy at 63X magnification.	
4.14	Mitochondrial membrane potential changes in control, PARP	87
	inhibitor treated and ADPRT1A OE (AOE) cells at 3 hrs and 5hrs	
	post 0.03mM cumene H ₂ O ₂ treatment.	
4.15	Phosphatidylserine (PS) exposure (Annexin V-FITC Staining) and	89
	PI staining in control and ADPRT1A OE (AOE).	
4.16	Role of ADPRT1A in D. discoideum development	90
4.17	Developmental expression pattern of ADPRT1A.	92
4.18	Densitometric analysis of PARP activation up to 6 hrs of nutrient	92
	starvation.	
Ch 5	5 : ADPRT1A knock-out and its possible role in <i>D. discoideum</i> growt	h and
	multicellularity	
5.1	Strategy for Sequential cloning of PARP (ADPRT1A) 5' and 3' HR	103
	into pDrive vector.	
5.2	PCR amplification of 5' and 3'HR regions of ADPRT1A	104
5.3	Screening of 5'HR-Bsr pDrive by RE digestion	104
5.4	Screening of 5'HR-Bsr-3'HR pDrive by RE digestion	105
5.5	RE digestion pattern for confirmation of <i>PARP</i> (ADPRT1A)	106
	knockout construct.	
5.6	RE digestion pattern of ADPRT1A knockout construct	107
5.7	Screening of random integrant knockout clone by PCR	108
5.8	Confirmation of knockout of PARP (ADPRT1A) by PCR at genomic	109
	level.	
5.9	Functional characterization of ADPRT1A KO	110
5.10	NAD ⁺ concentration in control and ADPRT1A KO cell using	110
	Bernofsky's Enzymatic recycling method.	
5.11	Growth analysis of Control and ADPRT1A KO cells:	111

5.12	Cell cycle analysis and CYCLINB transcript levels in ADPRTIA KO	113		
	cells:			
5.13	Effect of oxidative stress on ADPRT1A KO cells	114		
5.14	DNA damage in ADPRT1A KO	115		
5.15	Role of ADPRT1A in D. discoideum development	116		
5.16	cAMP estimation and chemotaxis assay	118		
5.17	Effect of exogenous cAMP pulsing on chemotaxis and development	120		
	in ADPRT1A KO cells			
Ch 6 :	Gene expression analysis of developmental genes involved in D. disc	coideum		
development				
6.1	Expression patterns of PDSA in ADPRT1A KO cells	134		
6.2	Expression patterns of PDSA in ADPRT1A OE cells	135		
6.3	Expression patterns of <i>PDSA</i> in PARP inhibited cells.	136		
6.4	Expression patterns of PDSA in PARP dR cells	137		
6.5	Expression patterns of REGA in ADPRT1A KO cells	138		
6.6	Expression patterns of REGA in ADPRT1A OE cells	139		
6.7	Expression patterns of <i>REGA</i> in PARP inhibited cells	140		
6.8	Expression patterns of REGA in PARP dR cells	141		
6.9	Expression patterns of ACA in ADPRT1A KO cells	142		
6.10	Expression patterns of ACA in ADPRT1A OE cells	143		
6.11	Expression patterns of ACA in PARP inhibited cells	144		
6.12	Expression patterns of ACA in PARP dR cells	145		
6.13	Expression patterns of CAR1 in ADPRT1A KO cells.	146		
6.14	Expression patterns of CAR1 in ADPRT1A OE cells.	147		
6.15	Expression patterns of CAR1 in PARP inhibited cells	148		
6.16	Expression patterns of CAR1 in PARP dR cells	149		
6.17	ADPRT isoforms in ADPRT1A KO	151		
6.18	Prespore-Prestalk markers in ADPRT1A KO slugs	152		
6.19	Neutral red stained slugs of Control and ADPRT1A KO cells	153		
Ch 7 : Conclusions				
7.1	ADPRT1A in D .discoideum growth	165		
7.2	ADPRT1A in D.discoideum development	166		