Synopsis of the

thesis on

Elucidating the role of Poly (ADP-ribose) polymerase in *Dictyostelium discoideum* growth and multicellularity

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Introduction:

The abundant nuclear enzyme PARP-1 is a multifunctional regulator of chromatin structure, transcription and genomic integrity and plays key role in a wide variety of processes in the nucleus. Poly (ADP-ribosyl)ation is a post-translational modification of proteins involved in the regulation of DNA metabolism, cell division, cell death and development (Kim *et al.*, 2005). PARP-1 influences ~3.5% of the total transcriptome of embryonic liver and stem cells and regulates ~60-70% of genes controlling cell metabolism, cell cycle and transcription. The role of PARP is majorly established as NAD^+ dependent modifying enzyme that mediates important steps in DNA repair, transcription and apoptosis, but its role during development is yet to be fully understood. Although**,** PARP1 is constitutively expressed, its enzyme activity is developmentally regulated (Kotova *et al*., 2009). PARP levels in a cell are under tight regulation and any disruption in PARP levels disturbs normal cell-functioning. PARP regulates critical gene transcription and cellular events. Regulated expression of *PRPA (PARP* ortholog) in *A. nidulans* was required for asexual development (Semighini *et al*., 2006). PARP is found to be distributed across all eukaryotes with notable exception of unicellular eukaryotes *S.cerevisiae* and *S. pombe* thereby suggestive of its probable role in multicellularity. Tulin and Spradling report that *PARP-1* deletion mutants in *Drosophila* fail to develop beyond larval stages due to defects in chromatin remodelling and regulation of gene expression (Tulin and Spradling, 2003). All these reports suggest that from a primitive/lower eukaryote *(A. nidulans)* to higher eukaryotes, PARP plays a crucial role in the process of development.

D. discoideum, a unicellular eukaryote exhibits multicellularity upon starvation and it has eight potential *PARP* (*ADPRT*) genes (Kawal *et al*., 2011). Among them only *ADPRT1A* and *ADPRT1B* possess the DNA binding domain *(in silico* studies). Inhibitors of PARP were extensively used to study the function of PARP. However these inhibitors have been reported to exhibit non-specific effects. Hence we used molecular genetic approach and our earlier studies on *PARP* (*ADPRT)* down regulation by antisense showed arrest in the development of *D. discoideum* (Rajawat *et al.,* 2011). A wide array of signaling molecules are involved in transition from growth to development in *D. discoideum* (Mir *et al.,* 2007). The present study thus deals with *in silico* analysis to identify the PARP1 ortholog in *D. discoideum* and also to understand its phylogenetic position. ADPRT1A- the PARP1 ortholog's overexpression and knockout studies in *D. discoideum* would help us to decipher the role of PARP in development. The study also

focuses on ADPRT1A's role in transcriptional regulation of genes required during *D. discoideum* developmental morphogenesis.

Objectives:

1. *In silico* analysis of PARP1 *(ADPRT1A)* of *D. discoideum.*
2. Over expression of *ADPRT1A* and its effects on *D. discoideum* growth and multicellularity.
3. *ADPRT1A* knockout and its possible role on *D. discoideum* growth and multicellularity.
4. Gene expression analysis of *ADPRT1A* and developmental genes involved in *D. discoideum* development.

Objective 1: *In silico* analysis of PARP1 (*ADPRT1A*) of *D. discoideum*

We have earlier reported eight potential *PARP* genes in *D. discoideum* (Kawal *et al.* 2011). Also, previous studies from the lab demonstrated PARP's role in development by down-regulation using antisense technique against the conserved catalytic domain and chemical inhibition of PARP (Rajawat *et al*., 2011). Results on constitutive *PARP* down-regulation did not show any effect on growth of the unicellular amoebae but interestingly development was blocked at initial aggregation stage while chemical inhibition led to delayed development. Of the 17 isoforms in humans, accumulating evidences suggest PARP1's probable role in differentiation and growth.

The study thus aims to illustrate the role of PARP1 in an unicellular eukaryote *D. discoideum*. *In silico* analysis was carried out to identify the PARP1 ortholog in *D. discoideum*. Protein sequences of known PARPs in Dictyostelium were obtained from *D. discoideum* database by searching for proteins containing the PARP catalytic domain. Eight protein sequences were retrieved from dictyBase and the phylogenetic tree was constructed by maximum likelihood method to identify the isoform closest to human PARP1. Of the eight isoforms identified, *ADPRT*2, *ADPRT1A* and *ADPRT1B* showed maximum similarity to human PARP1.The domain-wise analysis was done to identify the closest ortholog of human PARP1 in *D. discoideum* using the Pfam and Prosite. Unlike *ADPRT*2 and *ADPRT1B,* the predicted domain analysis of *ADPRT1A* alone showed presence of a 2 zinc fingers, pADPR1 domain, BRCT domain, WGR domain, PARP regulatory domain; which are key features identifying human PARP1. Also, multiple alignment of *ADPRT1A* with PARP from *H. sapiens, D. melanogaster, A.*

thaliana, and M. musculus by ClustalW showed the protein specified by the *ADPRT1A* transcript to have sequence homology to known PARP1 proteins. *ADPRT1A* possessed conserved features that define PARP1 viz., the metal binding residues of zinc finger 1, WGR motif, D loop residues, K893 for poly-ADP initiation and E988, the catalytic active site except a canonical caspase cleavage site (DEVD). Further phylogenetic analysis was done for *ADPRT1A* and *PARP* genes in 29 other members from various groups of protozoans, bacteria, lower and higher eukaryotes using the PHYLIP software.The treefile was constructed by TreeView and converted to an unrooted tree diagram using NJplot and it was seen that *ADPRT1A* was placed closer to humans as compared to plant *PARP* genes. *ADPRT1A* was thus identified as human *PARP1* ortholog in *D. discoideum*.

Objective 2: Over expression of ADPRT1A and its effects on *D. discoideum* growth and multicellularity

To generate *ADPRT1A* overexpressing *D. discoideum* cells, full length *ADPRT1A* (3046 bp) was PCR-amplified by using gene specific primers and cloned in *D. discoideum* expression vector act15/Acg-Eyfp and act15/Acg. The *ADPRT1A* clone was transformed into *D. discoideum* cells and maintained under G418 selection pressure to generate *ADPRT1A-Eyfp* and *ADPRT1A* overexpressing *D. discoideum* cells respectively. 60% increase in *ADPRT1A* transcript levels were found by Real time PCR thereby confirming *ADPRT1A* overexpression in *D. discoideum.* Further the localization of *ADPRT1A* was studied by checking for co-localization of YFP (C-terminal to *ADPRT1A*) in *ADPRT1A* OE *D. discoideum* cells and DAPI, a nuclear stain. Localization of YFP was consistent with that of DAPI indicating that *ADPRT1A* is a nuclear localizing protein.

PARP on activation utilizes NAD^+ as its substrate and thus resulting in depletion of NAD^+ pools (Kim *et al*., 2005). PARP activation was confirmed by estimating total NAD^+ from log-phase. *ADPRT1A* OE *D. discoideum* cells showed 60% reduction in NAD^+ levels as compared to control cells. These results also suggest that PARP is activated in *ADPRT1A* OE cells. *ADPRT1A* OE *D. discoideum* cells were comparatively slow growing with doubling time of approximately 18.48 ±1.65 hrs as compared to 12.41 ± 0.5166 hrs for control *D. discoideum* cells. This slow growth could be explained by reports on role of PARP in regulation of cell-cycle (Simbulan-Rosenthal *et al*., 2000). To explain slow growth of *ADPRT1A* OE cells, cell-cycle analysis was carried out by propidium iodide staining. *ADPRT1A* OE exhibited predominant population of cells in S and G2/M phase as compared to control cells at 48 hrs and 60 hrs (log-phase cells). Also,

ADPRT1A OE showed vacuole like structures which would be confirmed by TEM studies and results will be discussed in thesis.

To understand the slower growth, ROS levels were estimated in *ADPRT1A* OE cells. *ADPRT1A* OE cells showed higher endogenous ROS levels as compared to control. In addition, to confirm that all observations in *ADPRT1A* OE cells were not artefacts of high ROS but effects of PARP activation, growth profile of *ADPRT1A* OE were carried out in presence of benzamide, PARP inhibitor and N-acetyl cysteine, an antioxidant. Results clearly demonstrate that PARP inhibitor alone rescues the delay in growth of *ADPRT1A* OE cells while N-acetyl cysteine led to further slower growth; thus strengthening the role of *ADPRT1A* in growth of *D. discoideum.*

Depending on the signal, PARP either favors cell survival or cell death, thus deciding cell-fate. PARP1 acts to promote cell death in presence of extensive DNA-damage. Hence, to understand the effect of *ADPRT1A* OE on cell death, various cell death parameters were studied. Reduced NAD^+ levels suggested that PARP is activated in *ADPRT1A* OE cells. However, to validate PARP activity, PARP activation was seen by indirect immunofluorescence method at 2 min, 5 min and 10 min after 0.03mM H_2O_2 dose (paraptotic dose standardized in lab; Rajawat *et al*., 2014) in both *ADPRT1A* OE cells as well as control cells. *ADPRT1A* OE cells showed higher basal levels of PARP activation as compared to control and the activity peaked within 5 min. However, in control cells PARP activation peak was seen at 10 min. Also, PARP on activation generates ROS and thus resulting in oxidative damage. Therefore, levels of reactive oxygen species in *ADPRT1A* OE cells and in control cells were measured using DCFDA dye after treatment with 0.03mM H_2O_2 dose. ROS levels in *ADPRT1A* OE cells peaked within 10 min as opposed to 30 min in control cells. Mitochondrial membrane potential (MMP) loss is a characteristic feature of cell death and as PARP1 has a major role to play in cell-death, we hypothesize that overexpression of *ADPRT1A* may affect MMP in *D. discoideum* cells. MMP loss was checked using DiOC6 dye after subjecting *ADPRT1A* OE cells to oxidative stress of 0.03mM H_2O_2 dose. Log-phase *D. discoideum* cells were subjected to 0.03mM H_2O_2 dose for 3hrs and 5 hrs. *ADPRT1A* OE cells showed decreased fluorescence within 3 hrs suggesting complete loss of MMP while in control cells loss of MMP is observed at 5 hrs of 0.03mM H_2O_2 dose.

MMP changes were quantified using DiOC6 dye by FACS. Population of cells showing MMP loss post 3 hrs of 0.03mM H_2O_2 dose in *ADPRT1A* OE cells amounted to

approximately 81% out of which 40.1% cells had very less fluorescence intensity suggesting complete MMP loss. Control cells after 3 hrs of 0.03mM H_2O_2 dose did not show complete MMP loss. Next, we intended to check the mode of cell death in *ADPRT1A* OE using Annexin V-PI staining. *ADPRT1A* OE cells showed early Annexin V-FITC staining (due to early exposure of phosphotidyl serine) as well as early PI staining (1 hr and 3 hrs respectively).Control cells, on the other hand, showed Annexin V- FITC and PI staining at 3 hrs and 12 hrs respectively. Together, these results show that *ADPRT1A* OE cells are susceptible to oxidative stress as compared to control *D. discoideum* cells wherein 0.03mM H_2O_2 led to paraptotic cell death in control cells while the same dose led to necrotic cell death in *ADPRT1A* OE cells.

PARP is involved in a myriad of functions in the cell. Evidences demonstrate about its role in multicellularity and differentiation. PARP down regulation caused block in development at loose aggregate stage (Rajawat *et al.,* 2011). *ADPRT1A* OE cells also showed delay at aggregation stage (12-14 hrs) along with delay in both streaming (4-12 hrs) and early culminant stages (24-32 hrs) as compared to control cells.

Objective 3:ADPRT1A knockout and its possible role on *D. discoideum* growth and multicellularity.

The *ADPRT1A* gene was disrupted using the blasticidin cassette by homologous recombination in the genome and the transformants were selected using blasticidin-S as the selection pressure. The knockout strains obtained were first checked by appropriate PCR amplifications and further by Real Time PCR to check the transcript levels. ~93-96% decrease in transcript levels in *ADPRT1A* knockout *D. discoideum* cells was seen as compared to control cells. Effect of *ADPRT1A* KO on growth of *D. discoideum* cells was further studied. *ADPRT1A* KO *D. discoideum* cells showed slow growth with increase in the doubling time (24.95 ± 2.167 hrs) compared to control cells (12.26 ± 0.6703 hrs).To explain slow growth of *ADPRT1A* KO cells, cell-cycle analysis was carried out by Propidium iodide staining using FACS. It was observed that *ADPRT1A* KO *D. discoideum* cells at 48 hrs exhibited predominant population of cells in G2/M phase whereas control cell population was present in G0/G1 phase of the cell cycle at that particular time. Results clearly shows significant population of *ADPRT1A* KO cells in G2/M phase till 72 hrs of the growth. In order to know about the molecular mechanism happening during growth in PARP-1 inhibition or deletion condition, Simbulan-Rosenthal and their colleagues (2000) reported 40% gene alteration in

primary fibroblast *PARP* KO cells as compared to control cells. Interestingly, there were 2-3 fold down-regulations of *CYCLIN A* and *CYCLIN B1* in *PARP* KO cells. Reports also suggest that disruption of *CYCLIN A* expression or inhibition of *CYCLIN B* results in the cell cycle arrest at G2. Thus, to explain the G2-M arrest found in *ADPRT1A* KO, we analysed *CYCLIN B* gene expression and we found significant down-regulation of *CYCLIN B* ($p < 0.05$) in case of *ADPRT1A* KO cells and also PARP inhibitor treated cells. This result suggests that the absence of *ADPRT1A* caused slower growth due to G2-M cell cycle arrest and *CYCLIN B* down-regulation. Our study enlightens the role of *ADPRT1A* in cell cycle regulation in *D. discoideum*, thereby controlling the growth of *D. discoideum.*

ADPRT1A KO was confirmed by extraction of total NAD^+ from log-phase *ADPRT1A* KO *D. discoideum* cells which showed increase in NAD^+ levels as compared to control cells. These results suggest that *ADPRT1A* is indeed a major utilizer of NAD^+ in *ADPRT1A* KO cells. In addition, previous reports suggest that both neuronal and pancreatic islet cells of *PARP-1* KO mice are resistant to NO and other reactive oxygen species. *ADPRT1A* KO cells were found to be resistant towards oxidative stress (0.03mM Cumene H_20_2) compared to control cells indicating the absence of stress sensor, *ADPRT1A*.

The study also aimed to study the effect of *ADPRT1A* KO on development of *D. discoideum* cells, as earlier reports are indicative of its role in multicellularity. Conservation reports suggest that *PRPA* (a homolog of PARP-1) in *A. nidulans*) is present in all those filamentous fungi that form multicellular hyphae and developmental structures (Semighini *et al*., 2006). *ADPRT1A* KO cells also showed delay at aggregation stage as streaming was initiated after 8 hrs of starvation and fruiting body formation was late by 12 hours compared to control cells. cAMP signaling is required for aggregation to happen, therefore in order to explain the delay in the development. cAMP levels were estimated by direct ELISA method. There was significant decrease in the intracellular cAMP levels in *ADPRT1A* KO cells as well as PARP inhibitor treated and *ADPRT1A* OE cells compared to control cells. Both *ADPRT1A* OE and KO are leading to decrease in cAMP levels thereby explaining the delay in development. However, it is also suggestive of regulated levels of *ADPRT1A* being a requisite for proper D. *discoideum* development.

Objective4: Gene expression analysis of ADPRT1A and developmental genes involved in *D. discoideum* development

Studies have been associated with temporal changes in PARP activity, nuclear polymer concentration, and PARP expression (Tomoda *et a1*., 1991). We thus analysed the expression pattern of *ADPRT1A* during the growth and developmental phases by Real Time–PCR. The expression pattern of *RNLA* was also analysed as an internal control. The results obtained from the analysis demonstrated that *ADPRT1A* was expressed highest at the vegetative stage, loose aggregate and tight aggregate stage followed by significant reduction after aggregation stage *i.e* migrating slug, early culminant and fruiting body of *D. discoideum.* In addition, PARP activity was also studied and it was found to be higher in cells subjected to nutrient starvation from 2hrs till 6hrs. In order to explain developmental delay, genes which are required for the aggregation was analyzed using Real Time PCR wherein log-phase control, *ADPRT1A* KO, *ADPRT1A* OE and PARP inhibitor treated *D. discoideum* cells were starved and were kept at 22^{o}C for development. Cells were processed for total RNA isolation during initial aggregation stages that includes various time points like 0, 2, 4, 6, 8 hrs of development, followed by RNA quantification. Real Time PCR was performed in order to check the expression level using SYBR green method in terms of ΔCt values in Control cells and *ADPRT1A* KO, *ADPRT1A* OE and PARP inhibitor treated cells.

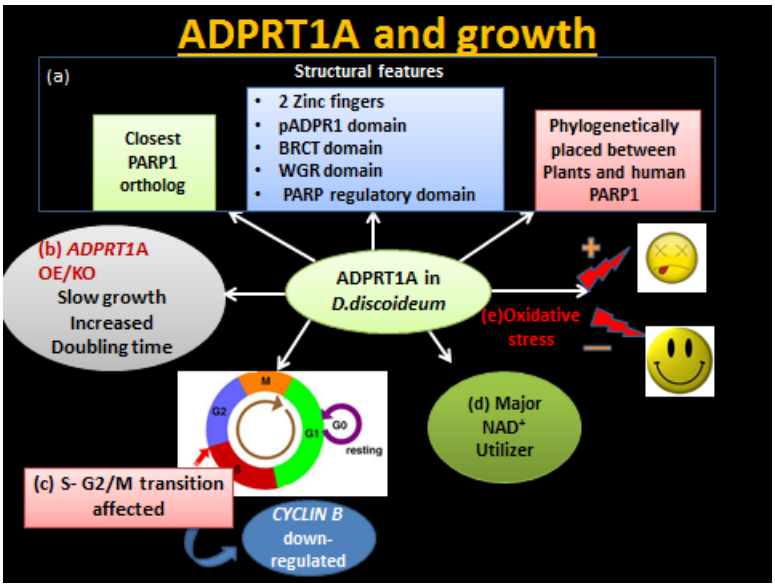
Numerous evidences report that disruption of any one of the genes involved in cAMP signaling such as *ACA, CAR1,* phosphodiesterases *PDSA* and *REGA* result in aggregation defects in *D. discoideum* and also the mutants that are defective in aggregate formation have defective expression of *ACA, CAR1, PDSA, REGA,* and *PDE4* (Parent *et al*., 1996).We analyzed the transcript levels of these following genes from 0-8 hrs of initiation of starvation which is the trigger for development in *D. discoideum. YAKA* gene expression was found to be significantly down regulated till 4 hrs of development in *ADPRT1A* OE cells compared to control. However, significantly higher levels of *YAKA* was observed in PARP inhibitor treated cells while no significant change was seen in KO cells after the onset of starvation. These results suggest the role of PARP catalytic activity in regulating YAKA levels which is essential for growth to differentiation transition as *ADPRT1A* KO did not lead to significant change in *YAKA* mRNA levels. Also, it is indicative of *ADPRT1A* acting in concert with other PARP homologs in the cell. *ACA* gene expression was also checked to explain lower cAMP levels. After the onset of starvation, no significant difference was seen in *ADPRT1A* KO cells. However,

ADPRT1A OE cells continued to show higher *ACA* levels at 8 hrs of starvation while PARP inhibitor treated cells showed vice versa i.e decrease in *ACA* mRNA. Next, we analysed the cAMP receptor 1. cAMP receptor 1 is expressed during aggregation and we analysed the levels of *CAR1* in *ADPRT1A* KO, OE and PARP inhibitor treated cells. Decreased levels of *CAR1* transcripts were found in *ADPRT1A* KO, OE and PARP inhibitor treated cells. However, sample size has to be increased for significant results which will be discussed in thesis. In addition, *PDSA* transcript levels were analyzed for understanding the role of ADPRT 1A during aggregation. *PDSA* (Extracellular phosphodiesterase) is vital in cAMP signaling as it maintains the cAMP gradient outside the cell and thus allowing proper signaling and hence aggregation. *PDSA* is up regulated by *YAKA* during initial hours of development. Significant increase in ΔCt values in *ADPRT1A* KO and PARP inhibitor treated cells was observed indicating *PDSA* transcript levels to be significantly lowered in *ADPRT1A* KO and PARP inhibitor treated cells which led to delayed development. In addition, PARP inhibited cells showed higher decrease as compared to *ADPRT1A* KO. Though PARP catalytic activity seems to be majorly contributing to its expression, physical presence of ADPRT1A may also be required for *PDSA* expression. *ADPRT1A* overexpression however, showed no change in *PDSA* transcript levels. *REGA being* a phosphodiesterase maintains intracellular cAMP levels. Significant decrease in ΔCt values in *ADPRT1A* OE cells from 2-6 hrs after starvation initiation was observed indicating *REGA* transcript levels to be significantly higher in *ADPRT1A* OE which led to decreased cAMP levels and hence delayed development. In contrast, *ADPRT1A* KO and PARP inhibitor treated cells showed no significant change in transcript levels.

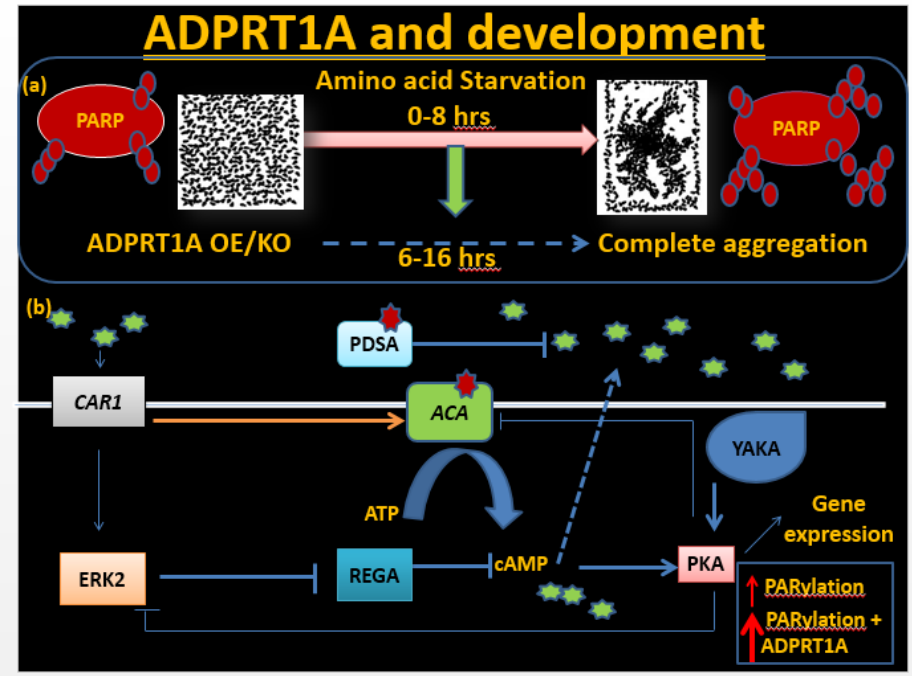
The developmental morphogenesis in *D. discoideum* involves differentiation of cells into prestalk and prespore cells which eventually form the stalk and spore respectively. PARP has been reported to play a crucial role in controlling gene expression of target genes involved in differentiation processes. With this line of thought, we intend to check the transcript levels of prestalk marker *ECMA, ECMB* and prespore marker *D19* in *ADPRT1A* KO, *ADPRT1A* OE and PARP inhibitor treated cells; which will be discussed in the thesis. Also, *ADPRT1A* containing plasmid would be inserted into *ADPRT1A* KO cells and rescue of the observed results in development and gene expression would be studied. This would be discussed in the thesis.

Conclusion:

PARP is a multifaceted protein having a myriad of roles in the cell. Out of the various roles of PARP, its role in cell differentiation and multicellularity has been not well studied. However, accumulating reports in the model systems like *D. melanogaster*, *A. nidulans* etc. suggest a probable role of PARP in growth and multicellularity. This study clearly shows regulated levels of PARP to be a requisite for *D. discoideum* growth and multicellularity. It has been found that absence of *ADPRT1A*, the PARP1 ortholog led to defects in both growth and development of the organism via regulation of cell cycle and the NAD^+ levels. Also, *ADPRT1A* is very essential during oxidative stress conditions leading to altered response in overexpression and knockout of *ADPRT1A*. The developmental morphogenesis is defective in both *ADPRT1A* overexpressing and knockout cells. PARP activity and PARP per se seems to be essential for proper functioning of the cAMP circuit thereby ensuring correct development.



ADPRT1A and growth: (a) ADPRT1A is identified as the closest PARP1 ortholog in *D. discoideum* with all key features defining PARP1 and it is phylogenetically placed in between plants and human PARP1 (b) ADPRT1A has been shown to affect the doubling time of *D. discoideum* (c) ADPRT1A is responsible for the S-G2/M transition of cell cycle via regulation of *CYCLINB* levels (d) ADPRT1A may lead to major NAD^+ consumption (e) In response to oxidative stress, ADPRT1A overexpression results in a susceptible phenotype while ADPRT1A knockout exhibits resistance to stress.



ADPRT1A and development: (a) Upon amino acid starvation, *D. discoideum* cells aggregate and undergo developmental morphogenesis. Concomitantly, significantly higher PARP activity was observed (b) Upon starvation, YAKA levels increase leading to increase in PKA activity which eventually leads to gene expression required for development. PKA also regulates the ACA (adenyl cyclase) and ERK2 levels. ERK2 inhibits REGA (intracellular phosphodiesterase) thereby regulating intracellular cAMP levels. Extracellular cAMP levels are maintained by *PDSA* (extracellular phosphodiesterase). ADPRT1A however controls *YAKA, ACA* and *PDSA* levels thereby regulating cAMP levels in the cell and hence correct aggregation process eventually leading to complete development of *D. discoideum. PARP* catalytic activity perse regulates *YAKA, ACA* levels while *PDSA* is affected by the presence of ADPRT1A.

References:

1. Kawal AM, Mir H, Ramniklal CK, Rajawat J and Begum R (2011). Structural and evolutionary analysis of PARPs in D. discoideum. *Am J Infect Dis* 7, 67–74.

2. Kotova E, Jarnik M and Tulin A (2009). Poly (ADP-Ribose) polymerase1 is required for protein localization to Cajal body. *PLoS Genet.* 5, e1000387.

3. Kim MY, Zhang T and Kraus WL (2005). Poly(ADP-ribosyl)ation by PARP-1: 'PARlying' NAD into a nuclear signal. *Genes Dev* 19:1951–1967**.**

4. Mir H, Rajawat J, Pradhan S and Begum R (2007) signalling molecules involved in the transition of growth to development in *Dictyostelium discoideum*. *IJEB.* 45,223-236.

5. Parent CA and Devreotes PN (1996). Molecular genetics of signal transduction in Dictyostelium. *Annu. Rev. Biochem.* 65, 411-440.
6. Rajawat J, Mir H and Begum R (2011) differential role of poly (ADP-Ribose) polymerase in *D. discoideum* growth and development. *BMC Dev. Biol*. 11, 14.
7. Rajawat J, Mir H, **Alex T**, Bakshi S, Begum R (2014). Involvement of poly (ADP-ribose) polymerase in paraptotic cell death of *D. discoideum. Apoptosis* 19, 90-101.
8. Semighini CP, Savoldi M, Goldman GH, Harris SD (2006). Functional characterization of the putative Aspergillus nidulans poly (ADP-ribose) polymerase homolog prpA. *Genet.* 173, 87-98.
9. Simbulan-Rosenthal CM, Ly DH, Rosenthal DS Konopka G, Luo R, Wang Z, Schultz PG and Smulson ME (2000). Misregulation of gene expression in primary fibroblasts lacking poly(ADP-ribose) polymerase. *PNAS*. 97, 11274-11279.
10. Tomoda T, Kurashige T, Yamamoto H, Fujimoto S and Taniguchi T (1991). Fluctuation of gene expression for poly (ADP-ribose) synthetase during homin-induced erythroid differentiation of human leukemia K562 cells and its reversion process. *Biochim. Biophys. Acta*, 1088, 359-364.
11. Tulin A and Spradling A (2003). Chromatin loosening by poly- (ADP)-ribose polymerase (PARP) at *Drosophila* puff loci. *Science* 299: 560–562.

PUBLICATIONS:

1. Rajawat J, Mir H, **Alex T**, Bakshi S, Begum R (2014). Involvement of poly (ADP-ribose) polymerase in paraptotic cell death of *D. discoideum. Apoptosis* 19(1): 90-101.
2. Rajawat J, **Alex T**, Mir H, Kadam A Begum R (2014). Proteases involved during oxidative stress induced poly (ADP-ribose) polymerase mediated cell death in *D. discoideum*. *Microbiol.* 160:1101-11.
3. Mir H, **Alex T**, Rajawat J, Kadam A and Begum R (2015). Response of *D. discoideum* to UV-C and involvement of poly (ADP-ribose) polymerase. *Cell Prolif*. 48(3):363-74.

Manuscripts under preparation:

4. **Alex T**, Kadam A, Saran S and Begum R. Poly (ADP-ribose) polymerase: a quintessential molecule for growth and multicellularity.

5. **Alex T**, Kadam A, Jariwala M, Bhatt S, Satyendra G and Begum R. Insights into the functional aspects of Poly (ADP-ribose) polymerase in growth and multicellularity.

Oral/Poster presented & conference/ workshop attended in Ph.D. duration:

1. **Alex T**, Kadam A, Lohia R, Saran S and Begum R." Poly ADP-ribose polymerase1 ortholog in Dictyostelium discoideum regulates its growth and development" at 'EMBO: Chromatin and Epigenetics-2015' held at EMBL, Heidelberg,Germany from 5 May 2015-10th May 2015 *(Awarded DST, CICS travel award)*

2. **Alex T**, Kadam A, Parikh N, Saran S and Begum R. "Fine tuning of Poly (ADP-ribose) polymerase is quintessential for cell survival" at 2nd Foundation Day of DBT-MSUB ILSPARE program held on 30th Sept, 2014 at MSU, Vadodara.

3. **Alex T**, Mir H, Rajawat J and Begum R. "Role of Apoptosis Inducing Factor in Mitochondrial Mediated Caspase Independent Cell Death in Dictyostelium discoideum" Cell symposia – Mitochondria: Signaling to Diseases, May 5-7, 2013 at Lisbon, Portugal. (Abstract accepted for Poster presentation)

4. **Alex T**, Mani S, Rajawat J, Mir H, Purohit V and Begum R. "Poly (ADP-Ribose) Polymerase :A link for the development of Dictyostelium discoideum." XXVI Gujarat Science Congress, Feb 26th 2012 at The M.S. University of Baroda, Vadodara.

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