Chapter 3:

In silico analysis of PARP-1 (ADPRT1A) of *D. discoideum*

Phylogenetic analysis of Poly (ADP-ribose) polymerase-1 in *D. discoideum*

3.1 Introduction

Poly (ADP-ribose) polymerase (PARP)-1 is a multifunctional nuclear protein belonging to the transferase family that catalyzes the formation of poly (ADP-ribose) polymers (PAR) (both linear and branched) on target proteins by utilizing its substrate, NAD^+ (Ame *et al*., 2004). In higher eukaryotes, PARylation is reversible through the action of PAR glycohydrolases (PARG), ARH3, Macro domain containing protein and NUDIX hydrolases (Uchida *et al*., 1993; Dunn *et al.* 1999; Rosenthal *et al.*, 2013). Poly (ADP-ribosyl) ation is a widely used post translational modification in eukaryotes and the occurrence of PARPs has been illustrated in all major eukaryotic groups (Citarelli *et al*., 2010; Perina *et al*., 2014). PARP-1 and PARylation influence a myriad of biological processes like DNA repair, transcriptional regulation, cell growth, differentiation and programmed cell death (Quenet *et al*., 2009; Hottiger *et al*., 2010; Messner *et al*., 2011; Mir *et al*., 2012). The role of PARP-1 is majorly identified as NAD^+ dependent modifying enzyme that mediates important steps in DNA damage response, transcription etc., however, its role during development and differentiation is yet to be fully understood. PARP homologs have been identified in protists, metazoans, filamentous fungi and plants with the notable exception of unicellular fungi, *S. cerevisiae* and *S. pombe* (Citarelli *et al*., 2010). There are a few reports suggesting that PARP-1 regulates critical gene transcription and cellular events during development. Thus the absence of PARP in unicellular eukaryote, yeast connotes plausible role of PARP in multicellularity and development.

D. discoideum is the simplest studied eukaryote that exhibits multicellularity (Raper, 1984) and it has eight potential *PARP* genes (Kawal *et al*., 2011). As per a previous study from our lab and another report, out of the eight isoforms, three are reported to be active (Rajawat *et al*., 2011; Couto *et al*., 2013). However, all eight isoforms show structural features for poly ADP-ribosylation but not for mono ADP-ribosyl transferase activity (Fey *et al*., 2004; Citarelli *et al*., 2010). *D. discoideum,* a social amoeba that is at the

transition of unicellular to multicellular forms of life, offers as an excellent model system to study the role of PARP in growth and multicellular development. We have investigated the role of PARP in stress induced cell death in *D. discoideum* (Rajawat *et al*., 2007; Mir *et al*., 2015). We have also shown PARP's role in *D. discoideum* development by inhibiting its basal PARP activity using Benzamide (PARP inhibitor) and delayed development was observed. We have also demonstrated that constitutive *PARP* down-regulation did not affect the growth of *D. discoideum,* nevertheless its development was found to be blocked at initial aggregation stage (Rajawat *et al*., 2011). Thus, our previous lab studies suggest that PARP plays an important role in *D. discoideum* development and cell death. However, we aim to pin-point the PARP family member out of eight isoforms that plays a vital role in *D. discoideum* development. Reports in other model systems suggest that PARP-1 to be essential for growth, development and multicellularity while PARP-2 has also been shown to play a compensating role (De Murcia *et al*., 2003; Semighini *et al*., 2006). Regulated expression of prpA (PARP-1 ortholog) was reported to be vital for asexual development for *A. nidulans*. Moreover, regulated expression of PARP was found to be essential for conidiospore development (Semighini *et al*., 2006). Also maximum accumulation of pADPr was observed at the pre-pupal stage of *Drosophila* development (Kotova *et al*., 2009). Thus, in view of the existing lab studies and reports on PARP's role in growth and development, the present study aims to identify the PARP-1 ortholog in *D. discoideum* and subsequently study its role in cell survival and multicellularity in the next chapters.

3.2 Results

3.2.1 Sequence similarity and phylogenetic analysis of PARP like proteins in *D. discoideum*

ADP-ribosylation is a well-studied post-translational modification that is involved in many cellular processes, including various signaling cascades, DNA repair, gene regulation and cell death (Gibson and Kraus, 2012). However, studies on it role in growth and differentiation remains elusive. Several aspects of the life cycle of *Dictyostelium discoideum* make it an attractive model to investigate the possible physiological role(s) of

ADP-ribosylation in growth and differentiation. PARP-1 contributes to 80-90 % of PARylation in cells (D'Amours *et al*., 1999). Thus, with this aim to identify the PARP-1 isoform in *Dictyostelium*, protein sequences of known PARPs in *Dictyostelium* were obtained from *Dictyostelium* (dictyBase) 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 (Dereeper *et al*., 2008) to identify the isoform closest to human PARP-1. Of the eight isoforms identified, *ADPRT*2, *ADPRT1A* and *ADPRT1B* displayed maximum similarity to human PARP-1 (Fig 3.1).

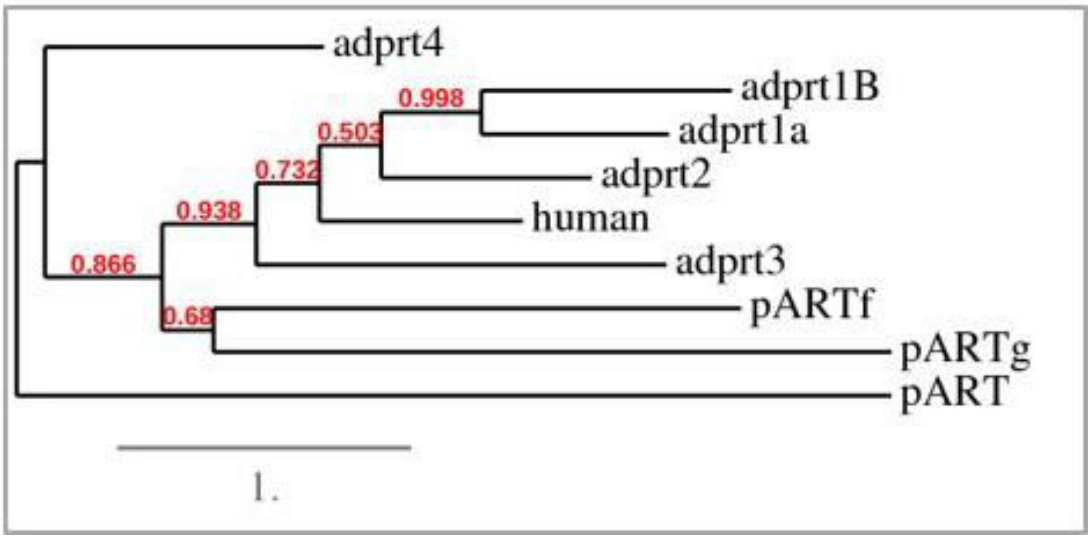


Fig. 3.1: Phylogenetic analysis of PARP isoforms in *D. discoideum* and human PARP by maximum likelihood method.

3.2.2 Domain wise analysis of ADPRT1A, ADPRT1B and ADPRT2 in *D. discoideum*

Further, domain wise analysis was carried out to identify the closest ortholog of human PARP-1 in *D. discoideum* using the Pfam and Prosite. The predicted domain analysis of *ADPRT1A* shows presence of 2 zinc fingers, pADPR1 domain, BRCT domain, WGR domain, PARP regulatory domain and the conserved PARP catalytic domain; which are

the key features of human PARP-1. However, ADPRT1B sequence shows presence of only 1 zinc finger, pADPR1 domain, WGR domain, PARP regulatory domain and the conserved PARP catalytic domain. BRCT domain characterized for protein-protein interactions were absent in ADPRT1B while ADPRT2 lacks zinc fingers and the pADPR domain (Fig. 3.2). Domain wise analysis proves ADPRT1A to be most similar to human PARP-1. In addition, the predicted positional organization for ADPRT1A as per domain analysis using the Pfam, Prosite and InterPro software was carried out as shown in Fig. (3.3A, B).

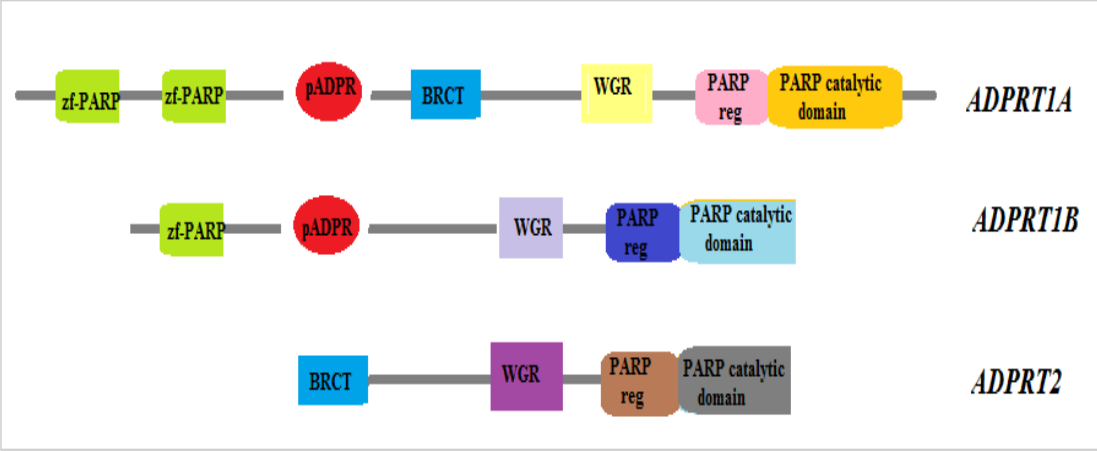
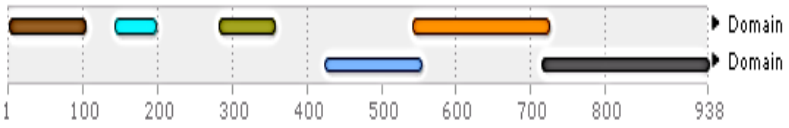


Fig. 3.2: Pictorial representation of domain organization of ADPRT1A, ADPRT1B and ADPRT2 by Pfam and Prosite.

(A)



(B)

Domains	Domain name	Position
	Zinc Finger	9-82
	pADR1	143-196
	BRCT domain	283-356
	WGR domain	461-542
	PARP regulatory domain	592-727
	PARP catalytic domain	716-938

Fig. 3.3: (A) Schematic representation of domain organization and (B) the positions in ADPRT1A by Pfam, Prosite and InterPro software.

3.2.3 Multiple alignments of ADPRT1A, ADPRT1B and ADPRT2 in *D. discoideum*

Furthermore, multiple alignment analysis of *ADPRT1A* protein sequence with PARP-1 sequences from *H. sapiens, D. melanogaster, A. thaliana* and *M. musculus* by Clustal W indicate that the protein encoded by *ADPRT1A* transcript have the definitive features of human PARP-1 *i.e.,* the zinc finger 1 of –CCHC ligand pattern, WGR motif, D loop residues, K893 for poly ADP initiation and E988 and the catalytic active site except a canonical caspase cleavage site (DEVD) (Fig. 3.4). *ADPRT1A* is thus identified as human PARP-1 ortholog in *D. discoideum***.**

3.2.4 Phylogenetic analysis of ADPRT1A and PARP-1 in other organisms

PARP and PARP-like proteins have been detected in bacteria, viruses, archaea and higher eukaryotes and it is validated that the complexity of PARP proteins has augmented with evolution (Jubin *et al*., 2016a). In view of this literature, a phylogenetic tree was created based on the multiple sequence alignment from 46 organisms (Fig. 3.5). Protein sequence alignments were

analyzed by *seqboot* in PHYLIP (VER. 3.6) to create 1000 boot strapped data sets. Distance between protein sequences was calculated by *Protdist* using Jones Taylor Thornton matrix of amino acid substitution. *Neighbor joining* was used to create 1000 phylogenetic trees, out of which a single consensus tree with bootstrap values was generated using *consense* tool. The tree showed ADPRT1A from *D. discoideum* to be closer to PARP-1 from *H. sapiens* as compared to plants. The order of sequence homology in descending order of similarity to human PARP-1 is as follows: *A. thaliana<D. discoideum<D. melanogaster<M. musculus<H. sapiens.* In conclusion, in our attempt to identify the PARP-1 ortholog in *D. discoideum*, ADPRT1A was identified to be closest to PARP-1 based on sequence similarity, domain organization and phylogenetic distance to human PARP-1.

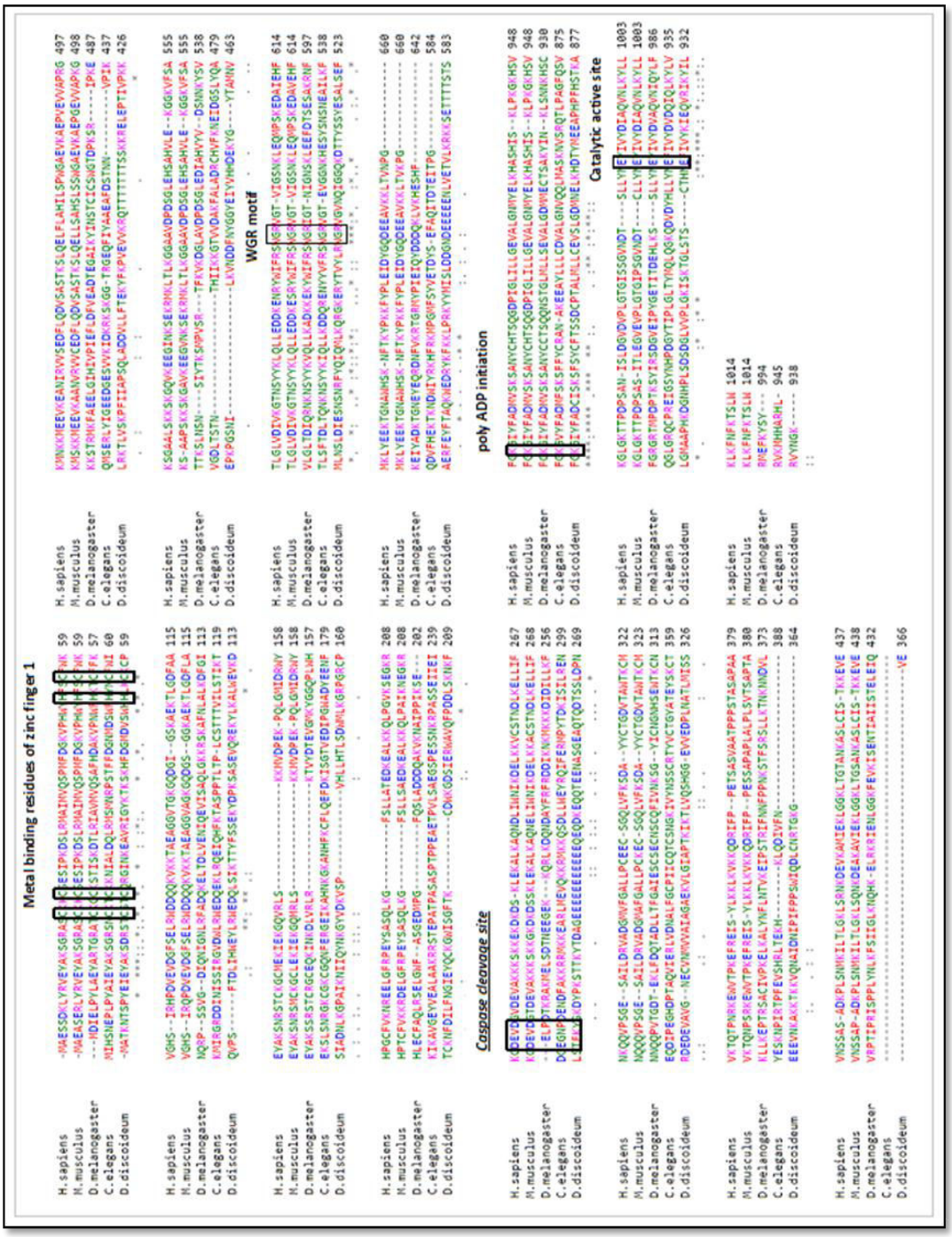


Fig. 3.4: Multiple alignment of protein sequences of PARP-1 from human and its orthologs from *M. musculus*, *D. melanogaster*, *C. elegans* and *D. discoideum* (ADPRT1A). ADPRT1A displays key features -CCHC- type zinc finger, WGR motif, poly ADP-initiation residues K893 and the catalytic active site.

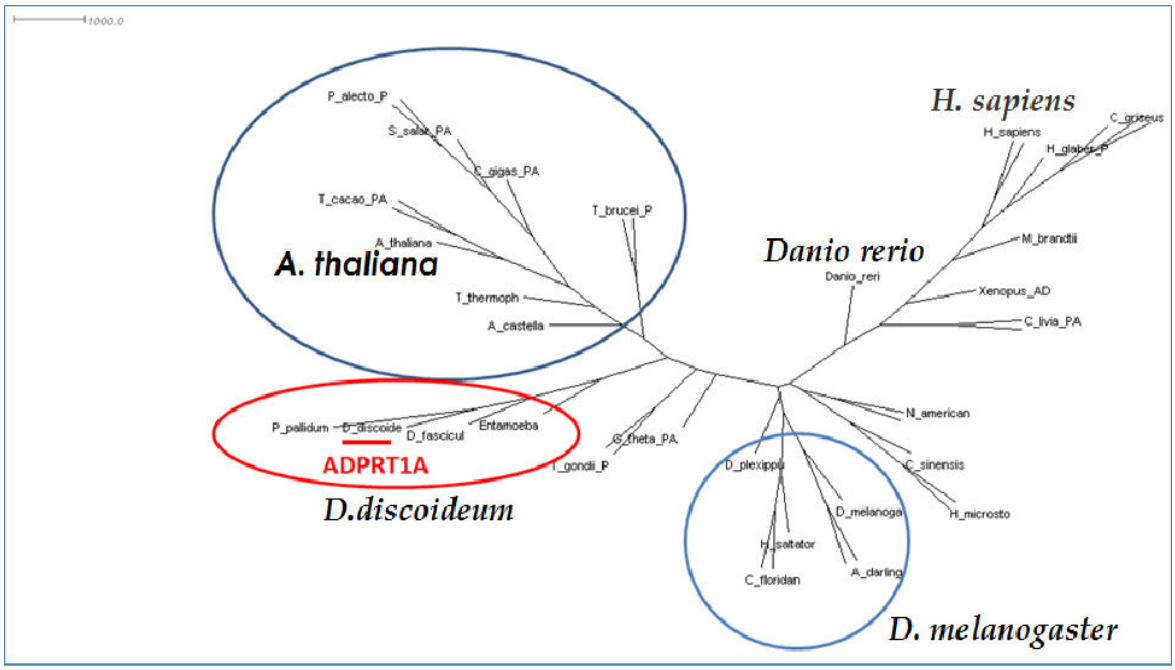


Fig. 3.5: Unrooted phylogenetic tree of PARP-1. Figure shows the branch length based on the alignment using CLUSTALW2. Neighbour joining (NJ) tree showing bootstrap values out of 1000, constructed using PHYLIP.

3.3 Discussion

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. Evidences across domain of life substantiate the role of PARP-1 from unicellular to multicellular forms (Jubin *et al.,* 2016b). *D. discoideum* is a unicellular eukaryote exhibiting multicellularity upon starvation and it has eight potential PARP (ADPRT) genes (Kawal *et al*., 2011). 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). In addition, PARP has also shown to be involved in stress responses like oxidative stress via proteases, UV-C stress via nitric oxide and DNA repair in *D. discoideum* (Rajawat *et al*., 2014 a, b; Mir *et al*., 2015; Couto *et al*., 2011). It

has been reported that PARP homologs exist in fungi that have multicellular hyphae and organized developmental structures and lack in yeast-like unicellular growth pattern (Semighini *et al*., 2006). However, reports over the last decade work on identifying the PARP family member which plays a vital role in development and multicellularity. The present study dealt with *in silico* analysis to identify the PARP-1 ortholog in *D. discoideum* and also to understand its phylogenetic position.

The *Dictyostelium* genome encodes eight PARP proteins (Kawal *et al*., 2011). *In silico* analysis and phylogenetic tree construction of PARP like proteins in *D. discoideum* identified three out of eight PARP isoforms to show highest similarity to human PARP-1. ADPRT1A and ADPRT1B branch out from the same node as seen in Fig.3.1 displaying more homology with human PARP-1. Kawal *et al*., also reported ADPRT1A and 1B to show maximum similarity to human PARP-1 by BLAST analysis while ADPRT2 was documented to be more similar to human PARP-2 (Kawal *et al*., 2011). Domain organization analysis showed only ADPRT1A to possess zinc fingers, BRCT domain, WGR domain and PARP catalytic domain (Fig. 3.2, 3.3). This analysis is in accordance with *ADPRT1A* domains defined by Couto *et al.,* (2011) using InterProScan. In addition, multiple alignment studies clearly confirm that *D .discoideum* may be a suitable model system to study PARP proteins. Multiple alignment results indicate that the protein encoded by *ADPRT1A* transcript have the definitive features identifying human PARP-1 *i.e.,* the metal binding residues of zinc finger 1 of –CCHC type (Eustermann *et al*., 2011), WGR motif involve, residues forming the D-loop (Wahlberg *et al*., 2012), K893 for poly ADP initiation (Simonin *et al*., 1993) and E988, the catalytic active site. However, the conserved DEVD caspase cleavage site was found to be absent in ADPRT1A (Fig.3.4). This could be due to absence of caspases in *Dictyostelium* (Roisin-Bouffay *et al.,* 2004). The two homologous zinc fingers (Zn1 & Zn2) in human PARP-1 are also characterized by a CCHC ligand pattern (Langelier *et al*., 2008; Langelier *et al.,* 2010). The human PARP-1 catalytic domain comprises of Glutamate residue at its 988 position (Schreiber *et al*., 2006). Thus analysis in the present study goes in accordance with the exiting literature for PARP-1 and thus proving *ADPRT1A* to be PARP-1 ortholog in *D. discoideum***.** Moreover, our phylogenetic analysis showed that ADPRT1A is a conserved

protein across domains and it has evolved as a unique protein in *Dictyostelium* and other related organisms. It shows homology to plants, fungus and animals. Phylogenetic tree obtained through neighbor joining method shows the position of *D. discoideum* after plants and protozoans but before the Ophisthokonta (animals and fungi) (Fig. 3.5). This position of *D. discoideum* as a whole is also in agreement with the previous reports (Eichinger *et al*., 2005).

3.4 References

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