Structural and Evolutionary Analysis of PARPs in *D. discoideum*

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Abstract: Problem statement: *Dictyostelium discoideum*, a unicellular eukaryote, exhibits multicellularity upon nutrient starvation, making it a better model for developmental studies and for the study of various signal transduction pathways. The most felicitous point of interest is that many of its genes show high degree of similarity to vertebrate genes. Poly (ADP-ribose) polymerase (PARP), a ubiquitous and abundant nuclear protein, has a number of distinct biochemical activities well suited for both structural and regulatory roles throughout its life cycle. We have analysed structural and evolutionary significance of PARP. Approach: *D. discoideum* lacks caspases and hence it exhibits caspase independent cell death which is of unique interest. PARP is a key protein involved in cell death in *D. discoideum*. An in silico approach to study the domain organization of PARP’s in *D. discoideum* would help us to understand evolution of the structural pattern from prokaryotes to eukaryotes. Results: Our previous studies showed involvement of PARP in *D. discoideum* cell death and development. We have attempted to probe the significance of PARPs in *D. discoideum* using bioinformatics approach. In this organism PARPs are encoded by 8 members whereas in *H. sapiens* there are 17 such members encoding PARP family. Conclusion: Our analysis suggests out of 8 genes, *adprt1a* and *adprt1b* seem to be involved in DNA damage response. Our approach with different bioinformatics tools suggests that these proteins also show homology with the *H. sapiens* counterparts. This article summarizes the domain organization of PARPs to throw light on the biological role of these proteins which will be helpful for further experimental studies in our model organism.

Key words: Poly (ADP-ribose) polymerase, *Dictyostelium discoideum*, structural homology, evolution, DNA damage response, Zn finger domain

INTRODUCTION

DNA damaging agents like ROS, MNNG and UV irradiation are known to activate PARP, a nuclear enzyme that has various physiological functions (Rajawat *et al.*, 2007; Burkle, 2001; De *et al.*, 1994; Lautier *et al.*, 1993; Shall and Murcia, 2000; Vodenicharov *et al.*, 2005). Activated PARP cleaves its substrate NAD⁺ and transfers ADP-ribose units to several target proteins including itself (Burkle, 2001; Shall and Murcia, 2000; Smulson *et al.*, 2000). Poly ADP-ribosylation is a unique post-translational modification playing crucial role in various cellular processes such as DNA damage signaling, repair, transcription regulation, chromatin modification, intracellular trafficking, mitotic apparatus formation and cell death. In response to DNA damage, PARP-1 uses NAD⁺ as a substrate and attaches polymers of ADP-ribose on different acceptor proteins (hetero-modification) or on PARP-1 itself (auto-modification), resulting into a branched polymer known as PAR (Poly ADP-ribose) which can be covalently linked mainly to glutamic acid residues (Hakme *et al.*, 2008) of acceptor proteins i.e., the polymerization starts at a glutamic acid residue (Skalitzky *et al.*, 2003). PAR moieties thus formed are degraded by Poly (ADP-ribose) Glycohydrolase (PARG) and lyase (Shunya *et al.*, 2006; Hayaishi and Ueda, 1982; Okayama *et al.*, 1978). Recently ADP-Ribosyl Hydrolase-3 (ARH3) in human has been identified to have PARG like activity (Mueller-Dieckmann *et al.*, 2003). The role of PARG-like activity of ARH3 seems to be not vital for cell death processes. Also, this enzyme does not significantly contribute to the cell survival process or PAR hydrolysis during cell stress conditions (Koh *et al.*, 2004).
Dictyostelium discoideum is a soil living amoeba that grows as separate, independent cells but interacts to form multicellular structures when challenged by adverse conditions such as starvation. Its genome consists of 34 Mb of DNA which is compacted into six chromosomes ranging from 4-7 Mb each (Eichinger et al., 2005). It comprises of nearly 8,000-10,000 genes and the most interesting point is that many of the genes show high degree of similarity with those of higher organisms. Structural studies with different bioinformatics tools revealed high homology of D. discoideum PARPs with those of H. sapiens. This article summarizes the domain organization of PARPs to throw light on the biological role of these proteins which will be helpful for further studies in our model organism.

PARPs in D. discoideum: Though the domain architecture analysis by PROSITE (Hulo et al., 2006; De et al., 2006), an ExPASy database of protein domains, suggests differences among H. sapiens and D. discoideum PARPs but their functions remain the same. It remains a matter of fascination that how PARPs interact with diverse set of proteins though functions of major domains have been dissected out. Also functions of a few domains like macro domain, WWE domain and WGR domains are still to be studied adequately. The only major difference observed on the evolutionary ladder is absence of Zn Finger (ZnF) in lower organisms (prokaryotes) while the transition from lower to higher forms of life the number of ZnF increases.

D. discoideum genes possess high degree of similarity with those of higher eukaryotes, here we demonstrate the domain homology of D. discoideum PARPs with that of H. sapiens. As shown in Table 1 in D. discoideum PARPs are encoded by 8 members whereas in H. sapiens there are 17 such members encoding PARP family. (Otto et al., 2005). BLAST (Altschul et al., 1990) analysis shows that ADPRT1A and ADPRT1B of D. discoideum show ~50% similarity to human PARP-1 (Fig. 1 and 2).

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Table 1: Representation of D. discoideum PARPs/pARTs (Kreppel et al., 2004; Fey et al., 2009)
ADPRT2 of *D. discoideum* is highly similar with PARP-2 of *H. sapiens* and the degree of similarity is described by providing BLAST results. Score = 365 bits (937), Expect = 3e-105, Method: Compositional matrix adjust. Identities = 228/572 (40%), Positives = 322/572 (57%), Gaps = 40/572 (6%).

Fig. 4: A, B, C demonstrate the ADPRT4 (*D. discoideum*), PARP-4 (*H. sapiens*) and BLAST results respectively. BLAST results Score = 154 bits (389), Expect = 3e-41, Identities = 155/613 (26%), Positives = 272/613 (45%), Gaps = 98/613 (15%).

These two proteins possess similar domains as human PARP-1 excepting being less by one ZnF domain. ADPRT2 of *D. discoideum* carries an extra BRCT (breast cancer susceptibility protein C terminus motif) domain and shares similarity to human PARP-2 by 57% (Fig. 3) whereas ADPRT4 which is similar to ADPRT2 in domain composition aligns better with human PARP-4, however ADPRT4 lacks VIT and VWFA domains (Fig. 4). ADPRT3, pARTg and pARTf also show different degree of homology with different human PARPs (Fig. 5-8). Table 2 summarizes the similarity between *D. discoideum* and human PARPs.

Representation of all the PARPs/pARTs present in *D. discoideum* with gene ID and protein ID sequence and it is fetched through the Dictybase (Kreppel *et al.*, 2004; Fey *et al.*, 2009) and EMBLmm (Guenther *et al.*, 1999), (Fig 1-8). Schematic representation of 8 different members of PARP superfamily of *D. discoideum* compared with that of *H. sapiens* PARPs.

It is well established that all the members of *D. discoideum* show high degree of similarity with that of higher eukaryotes.
This motif is also present in the sequence of ADPRT1A ZnF domain of *D. discoideum* (CX2C-X28/30-WHX2.......YEIEYKSDRSTCSTCQGINKAEVRIGYKTKSK HFDGMVSWHHLKCKCPQVPSFDTLHWEYLR WE...) (highlighted in red) hence this protein could also be involved in DNA damage sensing in *D. discoideum*. This BRCT domain is present in ADPRT1A, PARP-2 and PARP-4 in *D. discoideum*. This structure is modeled with template (2COK) Solution structure of BRCT domain of PARP-1 of Homo sapiens. The BRCT domain of PARP consists of AMD i.e., automodification domain. The automodification site comprises of ~9-15 glutamates residues which are thought to be important for automodification (Ikejima et al., 1990).

BRCT domain is also essential for protein-protein interaction which in turn is important for recruitment of XRCC1 to DNA damage sites in addition to the recruitment of PARG (necessary for PAR turnover) (D'Amours et al., 1999). WGR domain of PARP has been named after the most conserved central motif of the domain W-G-R as shown in the figure 10B and C. This domain is present in many of polymerases and other proteins with unknown function and ranges between 70-89 residues. The function of this domain is still unclear however it is proposed to be important in nucleic acid binding. The regulatory domain of the protein is in association with the C-terminal catalytic domain and consists of ~130 amino acids with duplication of 2 helix-loop-helix structural repeats. It is thought to relay the activation signal issued on binding to damaged DNA (Pion et al., 2005; Ruf et al., 1996; Oliver et al., 2004). Macro domain is ADP-ribose binding module (Karras et al., 2005). The 3D structure of the macro domain has a mixed α/β fold of a mixed β sheet sandwiched between four helices and consisting of ~180 amino acids. It has been suggested to play a regulatory role in ADP-ribosylation (Oliver et al., 2004). Catalytic domain possesses NAD⁺ binding site (Oliver et al., 2004). T coffee alignment results (Fig. 1) suggest that the catalytic domains of ADPRT1A, ADPRT1B, PARP-2, PARP-3, PARP-4, PARTf and PARTg have throughout conserved glutamate residue which is very essential for its activity. It has been reported that role of GLU 988 in human PARP catalytic domain is very important for its enzymatic activity (Gerald et al., 1995) hence the presence of GLU residue in catalytic domain of *D. discoideum* PARP reflects its function similar to that of human PARPs. Although proteins of the PARP family are related through their PARP catalytic domains, they may not resemble each other outside of that region.
Overlap of *D. discoideum* PARP with human PARP:
We used Superpose Web Server (Maiti et al., 2004) to obtain Root Mean Square Deviation (RMSD) in order to measure average distance and divergence between the backbones of superimposed domains of *D. discoideum* and *H. sapiens* PARP. RMSD reflects conformation of the protein backbone as well as the rotameric states of the side chains. Lower is the RMSD value better is the alignment of the superposed proteins.

Results suggest that the RMSD value of ADPRT1A for both the domains are between 0-3, whereas ADPRT1B shows higher RMSD for catalytic domain therefore ADPRT1A is more similar to that of human PARP-1 than ADPRT1B (Fig. 11).

**Evolution of PARP:** Using Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) (Jensen et al., 2009) we generated phylogenetic profile of PARP and related proteins i.e. organisms containing PARP and functionally related gene (Fig. 12). Functionally associated proteins often have similar phylogenetic profiles and conserved amino acids (Sanchez-Aguilar et al., 2007). It has been experimentally proved that this organism does not have caspases (Olie et al., 1998) which is also seen in the phylogenetic profile. Wet lab results in our lab have shown the involvement of PARP in *D. discoideum* cell death and development (Rajawat et al., 2007; 2011) which is substantiated by the appearance of PARP in *D. discoideum* in the phylogenetic profile. We have shown the involvement of PARP during *D. discoideum* normal development by our PARP down-regulation studies (Rajawat et al., 2011).

Constitutive PARP down-regulation resulted in blocked development while no effect was observed on *D. discoideum* growth (Rajawat et al., 2011). Interestingly, stage specific down-regulation arrested development at the slug stage (Rajawat et al., 2011). Also results in our lab have shown the role of PARP in oxidative stress and UV-C stress induced delayed development of *D. discoideum* (Rajawat et al., 2007; 2011).
ZnF as well as BRCT domain while BRCT domain is found in PARP protein in *A. nidulans* nevertheless, the ZnF domain remains absent here also. The appearance of ZnF domain in *D. discoideum* further signifies the transitional state of this organism between prokaryotes and eukaryotes. PARP shows an increase in the number of domains as well as number of encoding genes in the evolutionary lineage from lower organisms to higher eukaryotes. In higher organisms there is an addition of ZnF domain; *H. sapiens* contain three ZnF further signifying that there exists an evolutionary transition occurring in the PARP protein. The point of interest remains in the fact that it has been reported that the ZnFs are essential for DNA binding during DNA damage. However, absence of these ZnF in lower organisms is intriguing. It would be interesting to investigate the DNA damage sensing role of PARP in these organisms. Further in the evolutionary tree it has been observed that even though new domains are added, 70% homology in catalytic domain has been observed throughout the lineages.

All these results suggest that the evolution of this protein is directed such that the organisms become more efficient in linking DNA associated processes sensed by ZnF to other systems via protein-protein interactions through BRCT domain within a cell. In addition to the catalysing activity of PARG the presence of AMD in BRCT domain functions to refine the regulations of PARP.

**CONCLUSION**

Poly (ADP-ribose) polymerase in higher eukaryotes is known to be involved in DNA damage response. We have attempted to generate structure of the various domains as well as the protein folding of *D. discoideum* PARPs. *D. discoideum* PARPs show differential homology and domain structure and function. BLAST results show that ADPRT1A and ADPRT1B show maximum homology to *H. sapiens* PARP-1. Also overlapping studies of the catalytic domains of *H. sapiens* PARP and *D. discoideum* ADPRT1A and ADPRT1B depict remarkable resemblances. This study highlights the possibility of both these ZnF bearing proteins to be involved in DNA damage response like their mammalian counterparts. The phylogenetic profile and domain analysis highlight the fact that higher organisms possess more number of genes for PARP protein. This is further substantiated by differential results obtained by PARP inhibition in *E. coli*, *B. thuringiensis* and *D. discoideum* multicellular development. Overall this study points out that PARP protein has evolved to cope up the multitasking function along with the DNA damage response from unicellular prokaryotes to multicellular eukaryotes.

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