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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).

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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).

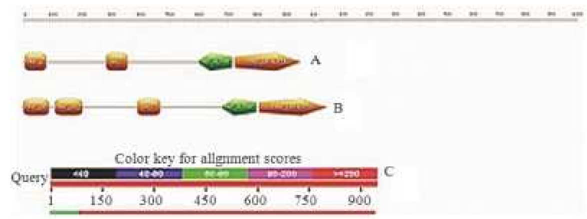


Fig. 1: A, B, C demonstrate the ADPRT1A *(D. discoideum)*, PARP-1 (*H. sapiens*) and BLAST results respectively. ADPRT1A of *D. discoideum* is highly similar with PARP-1 of *H. sapiens* and the degree of similarity is described by providing BLAST results. Score = 398 bits (1022), Expect = 1e-114, Method: Compositional matrix adjust. Identities = 306/975 (32%), Positives = 466/975 (48%), Gaps = 117/975 (12%)

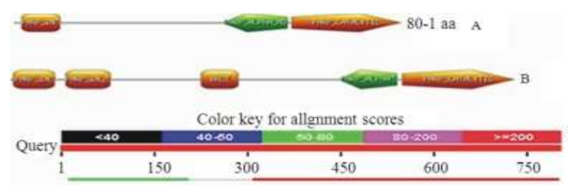


Fig. 2: A, B, C demonstrate the ADPRT1B *(D. discoideum)*, PARP-1 (*H. sapiens*) and BLAST result respectively. ADPRT1B of *D. discoideum* is highly similar with PARP-1 of *H. sapiens* and the degree of similarity is described by providing BLAST results. Score = 263 bits (672), Expect = 4e-74, Identities = 170/498 (35%), Positives = 263/498 (53%), Gaps = 43/498 (8%)

Table 1: Representation of *D. discoideum* PARPs/pARTs (Kreppel *et al*., 2004; Fey *et al*., 2009)

Aliases	Gene ID (dictybase)	Protein ID	Chromosome localization	Amino acids
Adprtla, PARP1, pARTa	DDB_G0278741	Q7Z115	Chromosome-3 1394183-1397588	938
Adprt2, PARP2, pARTc	DDB_G0292820	Q817C6	Chromosome-6 2124835-2127096	700
Adprt1b, PARP3, pARTd	DDB_G0279195	Q54X55	Chromosome-3 1 744004-1746597	804
Adprt 4, PARP4, pARTe	DDB_G0267468	Q7Z114	Chromosome-1 243053-245068	644
Adprt 3,TNKS, pARTb	DDB_G0291788	Q817C4	Chromosome-6 795337-803296	2536
pARTf, TNKS2	DDB_G0274389	Q86KC8	Chromosome-2 4856309-4858281	610
pART	DDB_G0289141	Q54HY5	Chromosome-5 2395095-2400678	1,803
pARTg, PARP14	DDB_G0271766	Q55AK6	Chromosome-2 860066-865156	1,618

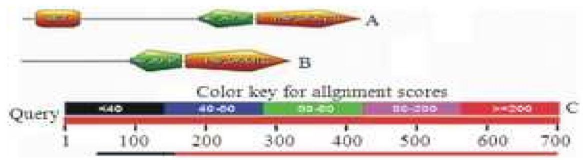


Fig. 3: A, B, C demonstrate the ADPRT2 *(D. discoideum)*, PARP-2 (*H. sapiens*) and BLAST results respectively. ADPRT2 of *D. discoideum* is highly similar with PARP-2 of *H. sapiens* and the degree of similarity is described by providing BLAST results. Score = 365 bits (937), Expect = 3e-105, Method: Compositional matrix adjust. Identities = 228/572 (40%), Positives = 322/572 (57%), Gaps = 40/572 (6%)

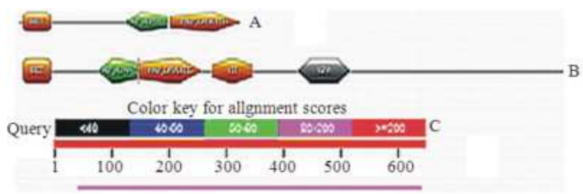


Fig. 4: A, B, C demonstrate the ADPRT4 *(D. discoideum)*, PARP-4 (*H. sapiens*) and BLAST results respectively. BLAST results Score = 154 bits (389), Expect = 3e-41, Identities = 155/613 (26%), Positives = 272/613 (45%), Gaps = 98/613 (15%)

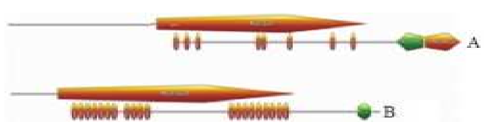


Fig. 5: A, B demonstrate the ADPRT3 *(D. discoideum)*, ANK17 (*H. sapiens*) which represent high degree of similarity of structural domains between them

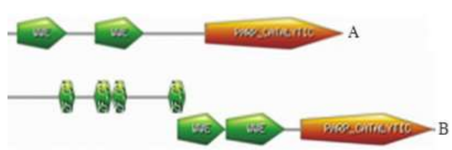


Fig. 6: A, B demonstrate the pARTf *(D. discoideum)* and PARP-12 (*H. sapiens*) respectively. The comparison of domains is shown

Fig. 7: A B demonstrate the pARTg *(D. discoideum)* and PARP-9 (*H. sapiens*) respectively. The comparison of domains is shown

Fig. 8: The figure demonstrates the domain structure of pART (*D. discoideum*).

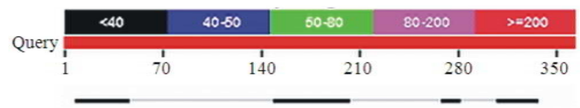


Fig. 9: BLAST result of *H. sapiens* ARH3 and *D. discoideum* ADPRH which shows E value 2e-05, Identities = 18/57 (32%), Positives = 30/57 (53%), Gaps = 3/57 (5%).

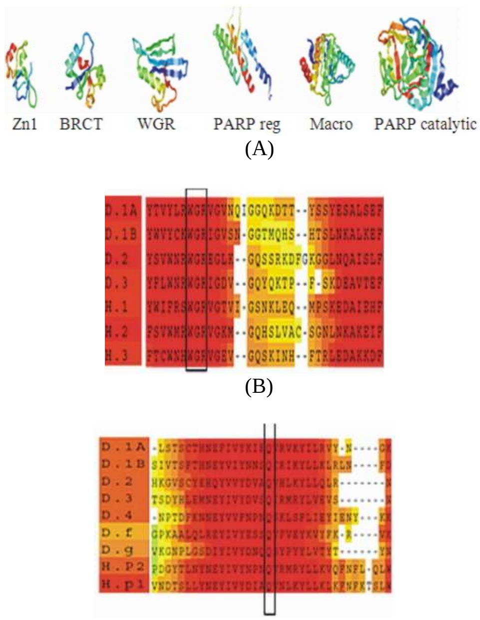
Table 2: Comparative analysis of *H. sapiens* and *D. discoideum* PARP domains

D. discoideum	*Homo sapiens*	Domains
ADPRT-1A and	PARP1	Zn finger
ADPRT-1B		BRCT
		PARP regulatory
		Catalytic
ADPRT 2	PARP2	BRCT
and PARP 4		PARP regulatory
ADPRT-3	ANK Repeat	Catalytic
	Domian 17	Ankyrin repeats
		PARP regulatory
pARTg	PARP 15	Catalytic
pARTf	PARP 9	Macro
		WWE
		Catalytic

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 (Guenter *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.



(C)

Fig. 10: (A) Folded structure of individual domains of *D. discoideum* PARP protein generated by SWISS MODEL (Arnold *et al*., 2006; Kiefer *et al*., 1995; Peitsch, 1995) (B) Multiple alignment of WGR domain sequence of PARPs from *D. discoideum* and *H. sapiens*. (C) Multiple alignment of catalytic domain of PARPs from *D. discoideum* and *H. sapiens*. This study (B and C) has been done by using T-coffee online server (Notredame *et al*., 2000)

Our BLAST search shows an ADPRH (*D. discoideum*) protein (Fig. 9) which shows ~53% similarity to (*H. sapien)* ARH3 protein which is shown to have PARG-like activity (Mueller-Dieckmann *et al*., 2003). Nonetheless the biological significance of PARG like activity of ARH3 is still poorly understood (Shunya *et al*., 2006).

Structural modeling of different domains of PARP in *D. discoideum*: As mentioned earlier certain domains shown in Fig. 10A form characteristic of PARP. Presence of Zn Finger (ZnF) domain is essential for sensing DNA damage and further recruiting DNA repair machinery. ZnF-like motifs of the form CX2C-X28/30-WHX2C are present in 1-3 copies at the N-terminus of different DNA repair enzymes (Caldecott *et al*., 1996; Mackey *et al*., 1999; D'Amours *et al*., 1999) hence PARP ZnF is associated with the function of strand-break sensing (Caldecott *et al*., 1996; Mackey *et al*., 1999; D'Amours *et al*., 1999; Gradwohl *et al*., 1990; Ikejima *et al*., 1990).

This motif is also present in the sequence of ADPRT1A ZnF domain of *D. discoideum* (CX2C-X28/30-WHX2……
..YEIEYAKSDRSTCSTCQRGINKEAVRIGYKTKSK
HFDGMDVSWHHLKCKCPQVPSFTDLIHWEYLR
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.

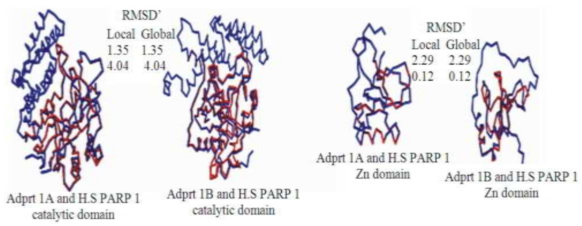


Fig. 11: Superimposition of catalytic domains and zinc finger of ADPRT1A, ADPRT1B with that of *H. sapiens* PARP-1

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).

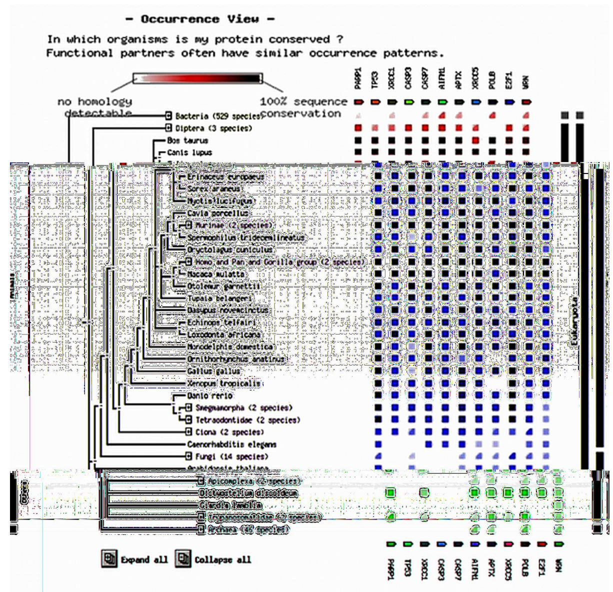


Fig. 12: Phylogenetic profile of PARP and related proteins

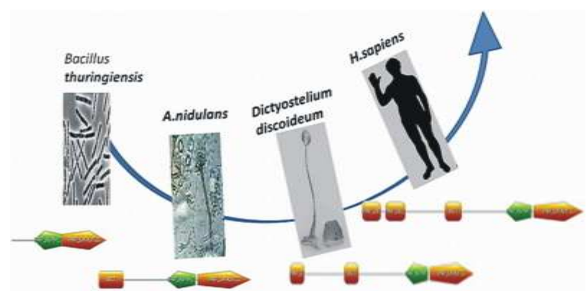


Fig. 13: Evolution of PARP protein

These results emphasize that PARP is essential for complex differentiation and its function may be linked to multicellularity adding a feather to its multitasking characteristic. On the other hand PARP inhibition during oxidative stress in lower organisms like *E. coli* and *B. thuringiensis* shows contrasting results. *E. coli* did not show any effect of PARP inhibition when subjected to oxidative stress unlike *B. thuringiensis* wherein inhibition of PARP rescued oxidative stress induced cell death. These results (data not shown) are in accordance of the phylogenetic profile (Fig. 12) as done using STRING server which shows *E. coli* lacks PARP whereas genome of Bacillus spp bears it.

With evolution, proteins gain and lose certain functions, this reflects in terms of gain and lose of domains corresponding to the functions. PARP protein also displays such evolution. The Fig. 13 depicts that PARP like protein of bacteria *B. thuringiensis* lacks

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 catabolising 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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