Conclusion

The abundant nuclear enzyme PARP-1 is a multifunctional regulator of chromatin structure, transcription, and genomic integrity and plays a 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 stem cells and regulates ~60-70% of genes controlling cell metabolism, cell cycle and transcription (Kraus 2008). The role of PARP-1 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. Recent findings have highlighted PARP-1 as a potential molecule involved in the process of growth and differentiation (Ji and Tulin 2010, Jubin *et al.,* 2016). However, its role in differentiation and multicellularity is yet to be well understood. PARP-1 is a constitutively expressed protein but its enzyme activity is reported to be developmentally regulated (Kotova *et al*., 2009). Also PARP-1 levels in a cell are under tight regulation and any disruption in PARP levels disturbs normal cell-functioning. PARP-1 regulates critical gene transcription and cellular events. It was seen that regulated expression of PRPA (PARP ortholog) in *A. nidulans* was required for asexual development (Semighini *et al.,* 2006). Tulin and Spradling also 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). In addition, 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. 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). Inhibitors of PARP have been 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). However, downregulation caused all PARP isoforms to be downregulated as the antisense was generated against the conserved catalytic domain, thus enabling us to understand the probable role of PARPs in development. However, the PARP family member contributing in controlling *D. discoideum* growth and development could not be identified. 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 PARP-1 ortholog's overexpression and knockout studies in *D. discoideum* would help us to decipher the role of PARP-1 in its growth and development. In addition, wide arrays of signaling molecules are involved in transition, from growth to development in *D. discoideum* (Mir *et al.,* 2007). The study also focuses on ADPRT1A's role in transcriptional regulation of genes required during *D. discoideum* developmental morphogenesis.

Our *in silico* analysis and phylogenetic tree construction of PARP like proteins in *D. discoideum* identified three out of eight PARP isoforms to show the highest similarity to human PARP-1. Domain organization analysis showed only ADPRT1A to possess zinc fingers, BRCT domain, WGR domain and PARP catalytic domain. Also, multiple alignment results with other well studied model organisms indicate that the protein encoded by *ADPRT1A* has the definitive features identifying human PARP-1 confirming that *D. discoideum* to be a suitable model system to study PARP proteins. 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 (Fig. 7.1a). This position of *D. discoideum* as a whole is also in agreement with the existing reports (Eichinger *et al*., 2005).

The present study also focused on understanding the role of the identified PARP-1 ortholog, *i.e* ADPRT1A for growth and development in *D. discoideum*. Our studies show that *ADPRT1A* overexpression (A OE) led to slow growth of *D. discoideum*(Fig 7.1b)

and significant population of AOE cells were in S and G2/M phase (Fig 7.1c). Also, AOE cells exhibited high endogenous PARP activity, significant NAD^+ depletion (Fig 7.1d) and also significantly lower ADPRT1B and ADPRT2 transcript levels. Moreover, AOE cells also exhibited susceptibility to oxidative stress. AOE also affected development of *D. discoideum* predominantly streaming, aggregation and formation of early culminant which are concomitant with reports on PARP's role in *D. discoideum* development (Fig 7.2). In addition, under developmental stimuli, increased PARP activity was seen along with developmentally regulated transcript levels of *ADPRT1A* during *D. discoideum* multicellularity (Fig 7.2a). The above mentioned work is published in the *Differentiation* journal. Subsequently, an *ADPRT1A* KO was also generated to confirm ADPRT1A's *'*role in *D. discoideum* growth and development.

PARP-1 has been reported to play a role in induction of G1 arrest and regulation of G2 arrest (Masutani *et al*., 1995). *ADPRT1A* KO showed slower cellular proliferation (Fig 7.1b), G2-M arrest via *CYCLIN B* downregulation (Fig 7.1c) and it also exhibited resistance to oxidative stress as opposed to susceptibility to oxidative stress by *ADPRT1A* OE (Fig 7.1e) thereby strengthening its role in DNA damage sensing. This was further illustrated by significantly higher DNA damage in ADPRT1A KO cells as compared to control cells. The knockout also showed delayed aggregation during development along with aberrant structures. This goes in line with reports of *PARP-1* gene alterations in fungus resulting in defective development and decreased life span (Semighini *et al*., 2006; Kothe *et al*., 2010; Muller-Ohldach *et al*., 2011). Hence, our results in the knockout background establish ADPRT1A's role during early development. Cyclic-AMP acts as the signaling molecule during *Dictyostelium* development particularly during aggregation and culmination (Kessin, 2001). Defects in cAMP synthesis and chemotaxis could lead to small aggregates and delayed aggregation (Tang and Gomer, 2008; Garciandia *et al*., 2013). This delay in aggregation was explained by genetic and chemical alterations in ADPRT1A levels and activity which revealed decreased intracellular and extracellular cAMP levels during aggregation as compared to control cells (Fig 7.2b). Chemotaxis

analysis showed defective cAMP chemotaxis in the *ADPRT1A* KO (Fig 7.2b). Exogenous cAMP pulses rescued the aggregation and chemotaxis defect in the *ADPRT1A* KO. Moreover, expression analysis of cAMP signaling genes revealed down-regulation of genes essential for cAMP production and signaling (Fig 7.2b). PARP-1 has also been reported in regulating cellular differentiation (Pavri *et al*., 2005; Rouleau *et al*., 2011; Jubin *et al*., 2016). The *Dictyostelium* developmental cycle exhibits differentiation of cells into prespore and prestalk cells (Jeremyn *et al*., 1989). *ADPRT1A* KO also affected pre-spore and pre-stalk gene expression wherein *ADPRT1A* KO showed pre-stalk tendency (Fig 7.2b).

In conclusion, this study clearly shows regulated levels of ADPRT1A to be a requisite for *D.discoideum* growth and multicellularity. Absence of ADPRT1A leads to defects in growth of the organism due to changes in NAD^+ levels, G2-M arrest in the cell cycle, subsequently exhibiting a stressed phenotype. ADPRT1A is also demonstrated to control the response of the cell to stress conditions particularly oxidative stress. ADPRT1A has been illustrated to be essential for the developmental program of *D. discoideum.* Our results show PARP activity and PARP per se seems to be essential for proper functioning of the cAMP circuit under starvation conditions thereby ensuring correct development in *D. discoideum.* ADPRT-1A might work in conjunction with cellular signals thus resolving the complex packaging and in turn controlling gene expression or it may interact with histones and transcription factors to control their activity at target gene promoters, ultimately affecting gene expression. These findings provide the first report of the regulatory function of ADPRT1A in growth, cAMP signaling during aggregation as well as differentiation in *Dictyostelium.* Also, these results open new avenues to understand the role of PARP-1 in differentiation in higher eukaryotes and thus enlighten PARP-1 as a potential therapeutant in gene regulation and differentiation associated diseases

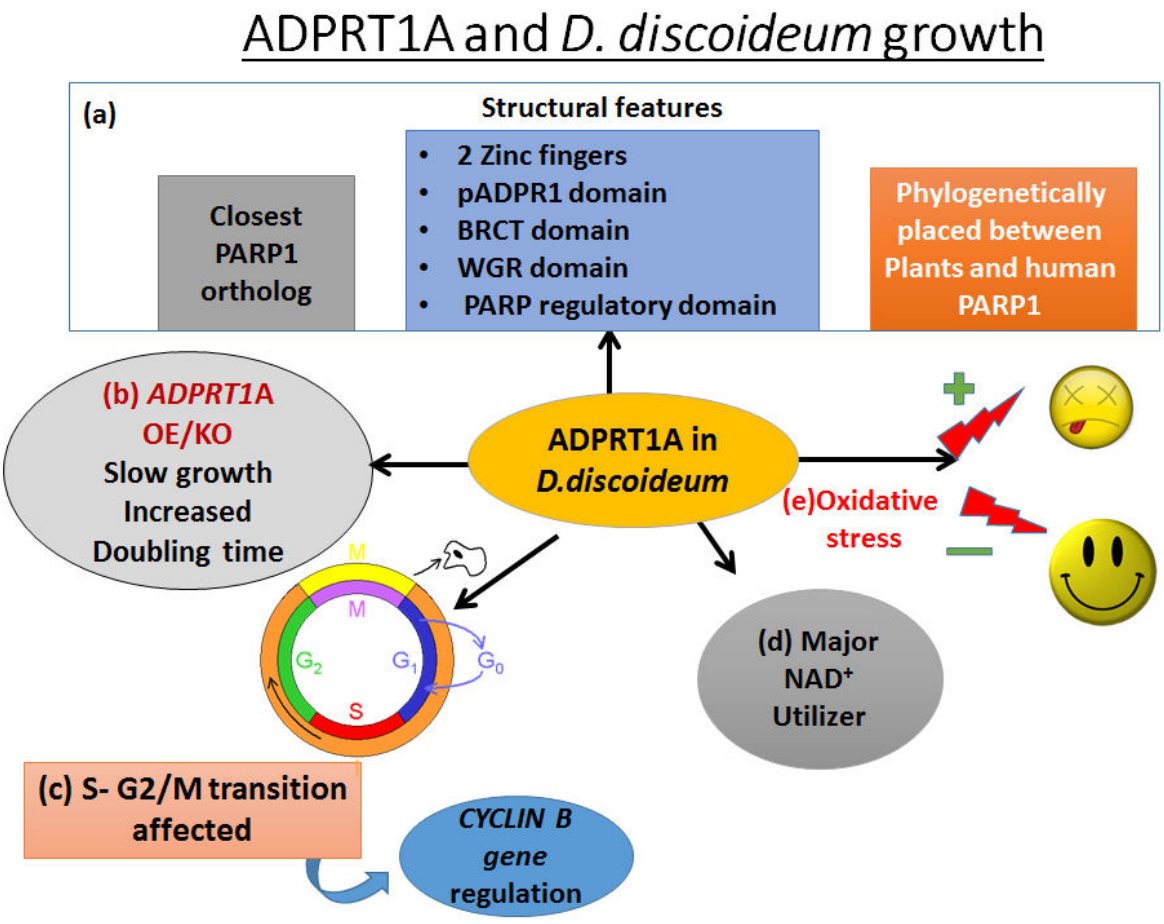

Fig. 7.1 ADPRT1A in *D .discoideum* growth. (a)ADPRT1A is identified as the closest PARP-1 ortholog in *D. discoideum* with all key features defining PARP-1 and it is phylogenetic position in between plants and human (b) ADPRT1A levels affect the doubling time of *D. discoideum* (c) ADPRT1A levels also affect the S-G2/M transition of cell cycle via regulation of *CYCLINB* levels (d) ADPRT1A leads to major NAD^+ utilization (e) In response to oxidative stress, *ADPRT1A* OE and *ADPRT1A* KO lead to susceptible and resistant phenotypes respectively.

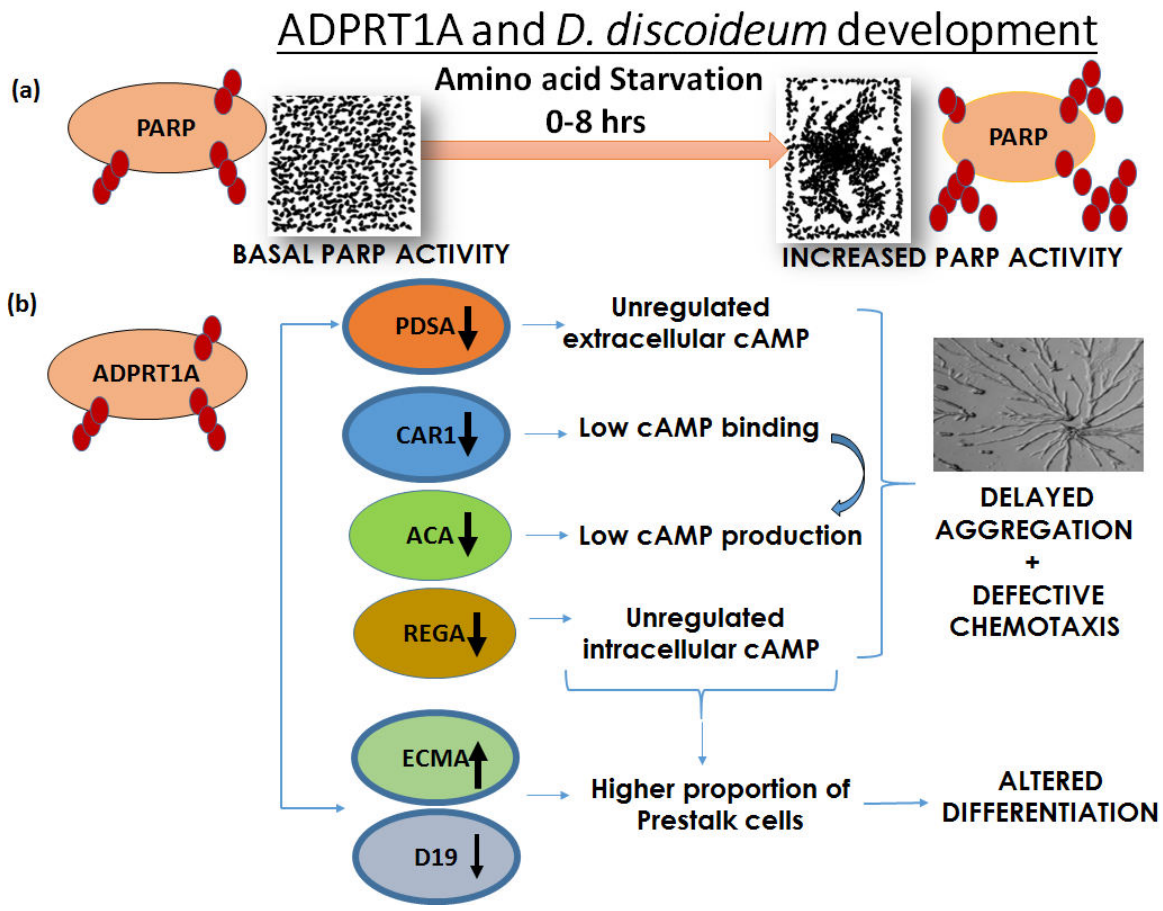

Fig 7.2. ADPRT1A in *D.discoideum* development. (a) Upon amino acid starvation, *D. discoideum* cells aggregate and undergo developmental morphogenesis. Concomitantly, significantly higher PARP activity is observed (b) *ADPRT1A* KO leads to lower a CAR1 transcript which leads to low cAMP binding resulting in reduced activation of ACA. Also *ACA* transcripts were also downregulated in absence of ADPRT1A. REGA (intracellular phosphodiesterase) regulates intracellular cAMP levels while extracellular cAMP levels are maintained by *PDSA* (extracellular phosphodiesterase) which are found to be downregulated thereby regulating the reduced cAMP levels in the *ADPRT1A* KO cells; the altered transcript levels thus lead to delayed aggregation and defective chemotaxis.

ADPRT1A also regulates differentiation markers in *D. discoideum.* Thus PARP catalytic activity as well as the ADPRT1A presence is essential for *D. discoideum* development.

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