Chapter 1:
Introduction

Accumulating reports over the decades demonstrate the most basic concepts of gene regulation (Naar *et al*., 2001). Cell growth and differentiation involves a complex and wide network of gene regulation mechanisms comprising of steps like initiation of cellular signaling pathways; activation and recruitment of transcription factors; chromatin modulation etc. However, a whole network of gene regulation machinery is necessary to promote and sustain these processes. One such an emerging family of proteins involved in gene regulation is the Poly (ADP-ribose) polymerase family.

1. The Poly (ADP-ribose) polymerase family

Poly (ADP-ribose) polymerase [PARP] enzymes belong to the transferase family of proteins involved in various cellular processes such as chromatin remodeling, DNA damage response, gene regulation, cell death etc. (D'Amours *et al*., 1999). ADP-ribosyltransferase D-type 1 [ARTD-1] catalyzes ADP-ribosylation of target proteins by transferring [ADP-ribose] units to carry out the elongation and branching of [ADP-ribose] chains (Lautier *et al*., 1993; D'Amours *et al*., 1999). These enzymes are present in all eukaryotes with a notable exception in unicellular yeast (Schreiber *et al*., 2006). The PARP family is a 17 member family of proteins possessing mono or poly [ADP-ribose] polymerase activity and a conserved catalytic domain along with various domains like zing finger, BRCT, SAM, SAP, Ankyrin and Macro domain (Ame *et al.,* 2004).

As per the new proposed nomenclature by Hottiger *et al.,* (2010) depending on their motifs and functions, the human PARP (hPARP) family is classified into three groups 1) PARPs 1-5: possessing conserved glutamate residue [(Glu988)]; 2) PARPs 6-8, 10-12 and 14-16: are putative mono [ADP-ribose] polymerases and 3) PARP 9 and 13 lacking PARP signature motif as well as Glu988 which suggests that they are inactive (Hottiger *et al*., 2010).

Out of the 17 members, PARP-1 [116 kDa] was the first characterized and extensively studied enzyme classically reported to play a vital role in DNA repair processes (Morales *et al*., 2014). PARP-1 and PARP-2 share ~69% homology in the catalytic domain and they are documented as vital proteins involved in DNA repair system (Schreiber *et al*., 2006) while PARP-3 was reported to be a mono-ADP ribosylating enzyme (Loseva *et al*.,

2010). PARP-2 and PARP-3 were considered as a subgroup of PARP-1 as they all carry out synthesis of branched polymers (Hassa *et al*., 2006). PARP-4, also known as Vault PARP, a ribonucleoprotein complex having poly [ADP-ribosyl] ation activity and it is involved in multidrug resistance of tumor and intracellular transport (Kickhoefer *et al*., 1999). Tankyrase-1 [TRF-1 interacting ankyrin-related [ADP-ribose] polymerase-1], also known as PARP5a, is identified to enhance telomere elongation by telomerase (Smith *et al*., 1998). Other PARP homologs show structural and functional differences. Tankyrase-2 lacks N-terminal HPS [His-Pro-Ser] domain, but it may share some overlapping functions with tankyrase-1 (Seimiya *et al*., 2006). Other PARP family members like tiPARP, PARP-12 and PARP-13 share PARP catalytic, WWE and CX8CX5CX3-like zinc fingers domain (Schreiber *et al*., 2006). The next subgroup which includes PARP-9/BAL1, PARP-14/BAL2/CoaSt6 and PARP-15/BAL3 are macro-PARPs, characterized by macro domains located before the PARP domain. This domain is found to be involved in transcriptional repression and X-chromosome inactivation, signifying its role as a transcription factor (Ma *et al*., 2001). The RNA recognition motif [RRM] and the Gly-rich domain of PARP-10 are known to help in binding of RNA with proto-oncoprotein, c-Myc (Schreiber *et al*., 2006). PARP-8 and 16 have recently been shown to be involved in assembly or maintenance of membranous organelles (Vyas *et al*., 2013). Functions of other members of the PARP family such as PARP-6 and PARP-11 are still elusive.

1.1 The Poly (ADP-ribose) polymerase-1

PARP-1 [EC 2.4.2.30] is the most prominent and well characterized member of the PARP family. It is a nuclear enzyme with approximately 10^6 copies per cell (Kameshita *et al.,* 1984) and accounts for 80-90% of total cellular PARylation. It has been implicated in various cellular processes such as DNA repair, transcription, cell death etc. (Jubin *et al.* 2016b).

1.2 PARP-1 Structure

PARP-1 is also the most structurally explored member of the family comprising three functionally distinct domains as shown in Fig.1.1: amino-terminal DNA Binding Domain (DBD), an auto-modification domain (AD) and carboxy-terminal Catalytic Domain

(CAT) which is accountable for the formation of Poly (ADP-Ribose) polymer (D'Amours *et al*. 1999; Krishnakumar and Kraus 2010).

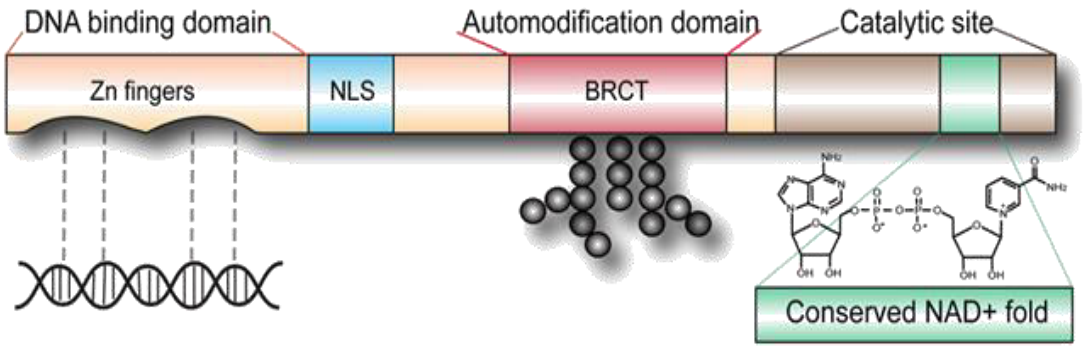

Fig. 1.1: PARP-1 protein structure containing three domains. PARP consists of the binding domain, Automodification Domain, Catalytic Domain with conserved NAD^+ fold (David *et al.,* 2009).

A. DNA binding Domain (DBD) - DBD contains three Zn fingers (FI, FII and FIII) which are structurally and functionally unique. It contains a bipartite nuclear localization signal (NLS) with which PARP-1 is targeted to the nucleus (Schreiber *et al.,* 1992). The two homologous zinc finger proteins (Zn1 & Zn2) are characterized by a CCHC ligand pattern (Langelier *et al*., 2008; Langelier *et al.,* 2010). Zn1 has been demonstrated to relay the signals to the catalytic domain for formation of PAR molecules (Langelier *et al*. 2008; Eustermann *et al*., 2011) while Zn2 is majorly involved in DNA binding as compared to Zn1 due to its higher affinity to DNA (Langelier *et al*., 2011). Eustermann *et al*., (2015) have demonstrated very recently how the two zinc fingers recognize SSBs and coordinate domain folding in PARP-1 to control the activity of the C-terminal catalytic domain. On the other hand, Zn3 has been reported to mediate inter domain contacts required for DNA dependent conformational change of PARP-1 (Langelier *et al*., 2010).

B. Automodification Domain- The automodification domain contains a BRCA1 C terminus like (BRCT) motif reported to be involved in protein-protein interactions, as well as multiple glutamate residues that are likely targets for auto-Poly (ADP-Ribosylation). Automodification can inhibit PARP-1 DNA binding, protein-protein interactions, and ADP-ribosyl transferase activity, eventually inactivating the protein (D'Amours *et al.,* 1999).

C. Catalytic Domain- The Catalytic Domain (CAT) catalyzes three different enzymatic reactions:

1. Initiation- Attachment of first ADP-ribose moiety to an acceptor amino acid
2. Elongation- Further addition of ADP-ribose moiety to pre-existing ones
3. Branching- Generation of branching points (Ruf *et al.,* 1998).

One contiguous ~50 amino acid sequence in the catalytic domain termed as the "PARP signature" motif (859 – 908 amino acids in hPARP-1) forms the active site. It shows 100% conservation among vertebrates and 92% conservation among all species, suggesting a critical role for PARP-1 activity in cellular function (Kraus and Lis, 2003). The 'PARP signature motif' also contains the NAD^+ acceptor sites and Glutamate residue at its 988^{th} position involved in PARylation (Schreiber *et al*., 2006). In addition to this, it also contains the highly conserved amino acid sequence *i.e.* Trp, Gly and Arg, termed as the WGR domain but its role is yet to be identified (Schreiber *et al*., 2006; Ye´lamos *et al*., 2011). However, Langelier *et al*., (2012) showed that Zn3 along with Zn1 and WGR domain of PARP-1 together bind to the damaged DNA leading to conformational changes ultimately leading to condensing the damaged site nearer to the catalytic domain. The structural organization of PARP-1 is depicted in Fig. 1.2.

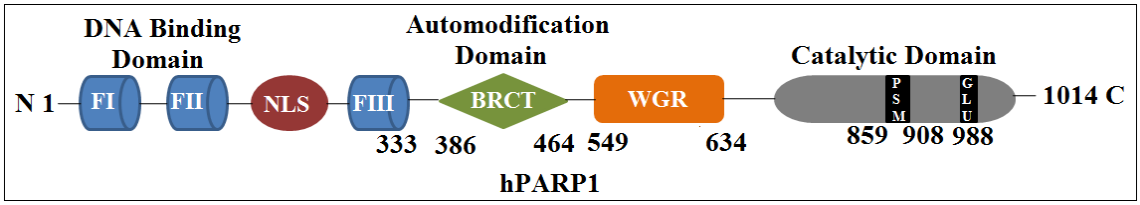

Fig 1.2: Structural organization of human PARP-1 (hPARP-1): It is characterized by FI, FII: Zinc finger motifs, FIII: Zinc ribbon domain (1-333 aa); NLS: Nuclear localization sequence; BRCT: BRCA1 C terminal motif (386-464 aa); WGR domain (549-634 aa) and the most conserved catalytic domain with PARP signature motif (PSM) between 859-908 aa and Glutamate (Glu) at 988 position (Jubin *et al*., 2016b).

1.3 PARP Activation

PARP activity has been found ubiquitously in organisms extending from archaebacteria to mammals with apparent absence in yeast (Hassa and Hottiger, 2002). PARP-1's basal enzymatic activity is very low, but it is dramatically enhanced by a variety of allosteric activators including single- and double-strand breaks, crossovers, cruciforms (D'Amours *et al.* 1999; Oei and Shi 2001; Kun *et al.* 2002, 2004; Kim *et al.* 2004; Lonskaya *et al.,* 2005) and supercoils, as well as some specific double-stranded sequences, post translational modification like phosphorylation (Burkle and Virag, 2013), environmental/developmental stimuli (Pinnola *et al.,* 2007) etc. There are reports suggesting that PARP-1 activation by single stranded breaks (SSBs) requires presence of both the zinc fingers-Zn1 and Zn2 while only Zn1 is required for double stranded breaks (DSBs) (Langelier *et al.,* 2008). PARP-1 has also been reported to have affinity for undamaged DNA structures as well as specific octamer motif "RNNWCAAA" found in several gene promoters (Ko and Ren, 2011).

Another alternative DNA independent mode of PARP-1 activation is based on kinase cascades. Phosphorylated ERK2 has been shown to significantly augment PARP-1 catalytic activity in the presence or absence of damaged DNA (Kauppinen *et al.,* 2006; Cohen-Armon *et al*., 2007). Interaction between PARP-1 and a pre-phosphorylated kinase has also been shown to mediate PARP-1 activation (Cohen-Armon, 2007; Cohen-

Armon *et al*., 2007). Similarly, during neuronal development, activated calcium-dependent protein kinase [CaMKII] phosphorylates PARP-1 enzyme leading to its activation subsequently promoting the nuclear export of its negative regulator KIF4 (Ju *et al*., 2004). Furthermore, protein phosphatase 5 [PP5] overexpression is also capable of enhancing PARP-1 enzymatic activity in response to double stranded DNA breaks (Dong *et al*., 2010). Nuclear Nicotinamide mononucleotide adenylyl transferase 1 [NMNAT1], an enzyme involved in NAD^+ synthesis also associates with PAR to enhance PARP-1 enzyme activity (Zhang *et al*., 2012). Other proteins regulating PARP-1 activity include Ku (Dong *et al*., 2010), histone variant mH2A1.1 (Ouararhni *et al*., 2006), KIF4 (Midorikawa *et al*., 2006), mono ADPribosylation by SIRT6 (Mao *et al*., 2011). Developmental or environmental stimuli too induce PARP-1 activation and PAR-dependent nucleosome loosening leading to histone stripping and transcriptional activation. This mechanism was studied in polytene-chromosome puffs of larval salivary-glands (Tulin and Spradling, 2003). Hence, PARP-1 can be activated by DNA dependent and independent manner which is summarized in Fig 1.3.

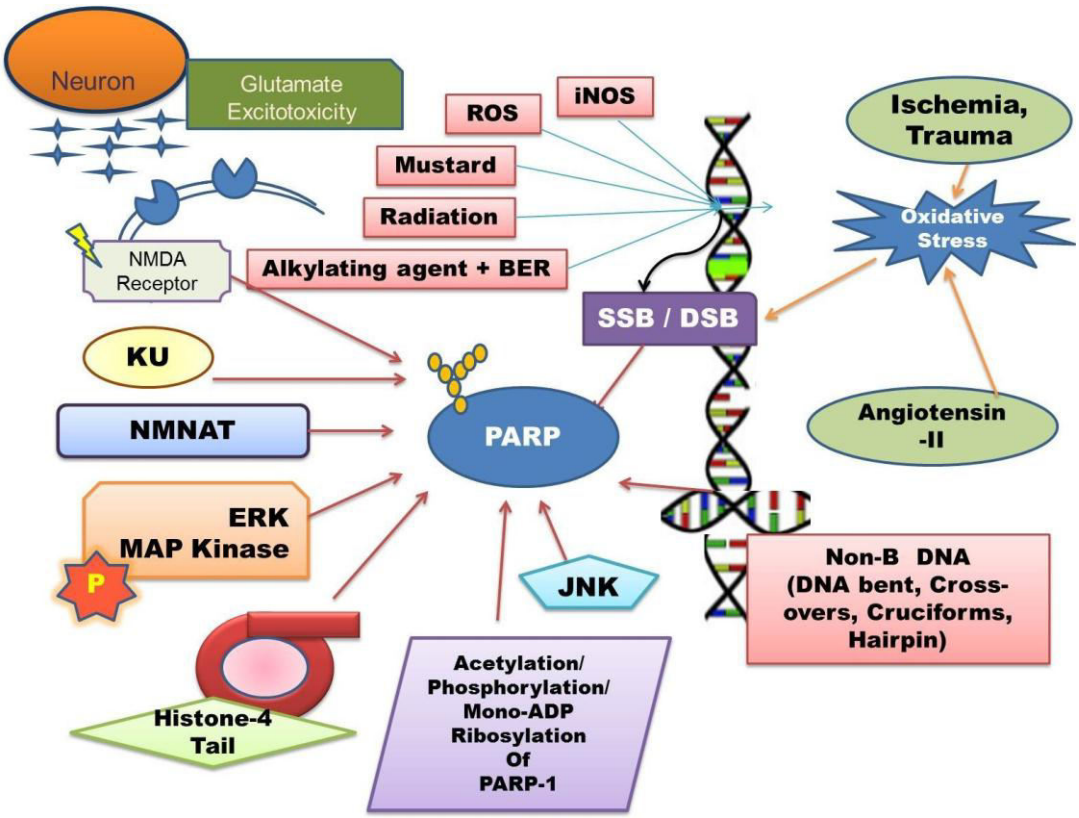

Fig. 1.3: Mechanism of PARP-1 Activation. The nuclear enzyme PARP-1 can bind to DNA breaks resulting in its activation. DNA breaks are caused either by ROS, RNS or radiation or indirectly by DNA repair machinery where breaks are introduced into the DNA strands as in the case of alkylating DNA damage. Binding to special non-BDNA structures such as cruciform or bent DNA or junctions may culminate into PARP-1 activation. DNA independent mechanisms like protein–protein interactions or covalent modifications (e.g. acetylation or phosphorylation, mono-ADP-ribosylation,) have also been described as activation mechanisms for PARP-1. Other proteins activating PARP include Nuclear NMNAT, Ku and phosphorylated ERK2 and Histone-4 tail (Jubin *et al*., 2016b).

Poly (ADP-ribose) glycohydrolase (PARG) catalyzes the hydrolysis of PAR producing free mono and oligo (ADP-ribose) via endo and exoglycosidic modes of action. ADP-ribosyl protein lyase cleaves the final ADP-ribose unit from the target protein thus releasing ADP-3-deoxypentose-2-ulose (ADP-DP) (Oka *et al*. 1984).

In vivo, the steady-state levels of PAR are maintained by the activities of PARPs and PARG. The degradation of PAR may begin immediately on the initiation of PAR synthesis and terminates completely within minutes after the cessation of PAR synthesis has occurred (D'Amours *et al.* 1999; Tulin and Spradling 2003). PARP-1 is present at a five-fold to 20-fold molar excess over PARG in some cell types, a variety of regulatory mechanisms thus act to control the levels of PAR in the nucleus (Alvarez-Gonzalez *et al.* 1999; D'Amours *et al.* 1999; Davidovic *et al.* 2001). PAR hydrolase (ARH3), which shares catalytic domain similarity with PARG also degrades ADP-ribose units (Oka *et al.* 2006). However, ARH3 does not hydrolyze ADP-ribose-arginine, -cysteine, -diphthamide, or -asparagine bonds (Oka *et al.*, 2006). In addition, Macro domain containing proteins and another set of enzymes viz., NUDIX hydrolases have also been reported to be involved in PAR degradation (Rosenthal *et al.*, 2013; Dunn *et al.*, 1999).

1.4 Functions of PARP-1

PARP-1 is involved in various functions therefore leading to increased complexity in understanding each of its functions. PARP-1 has been implicated in various biological and cellular processes like DNA damage detection and repair, cell death pathways, chromatin modification, transcription, etc. as shown in Fig. 1.4.

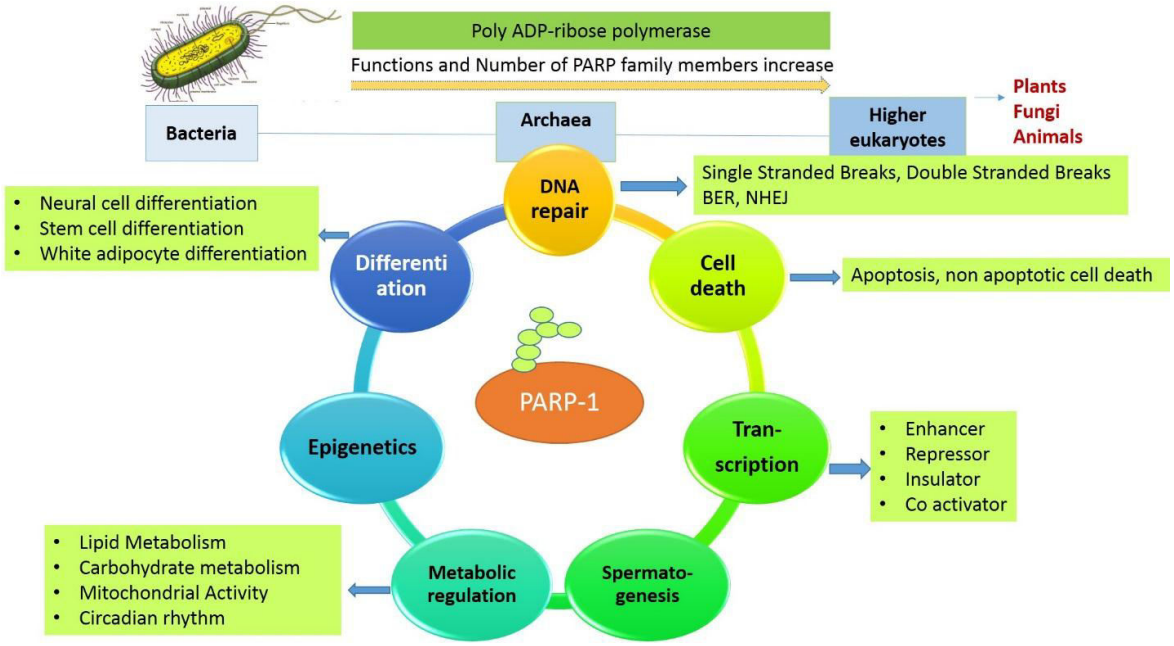

Fig. 1.4: Multifunctional roles of PARP-1. Poly ADP-ribose polymerase family of proteins is detected from prokaryotes to eukaryotes. The number of PARP family members and their involvement in various cellular processes increases with the complexity of the organism. PARP-1, the most studied PARP family member is involved in a wide range of processes like DNA repair, cell death, transcription, spermatogenesis, metabolic regulation, epigenetics and differentiation (Jubin *et al*., 2016b).

1.4.1 PARP-1 in DNA repair

ADP-ribosylation activity of PARP-1 is an instantaneous biochemical response to DNA damage induced by ionizing radiations, alkylations etc. At low levels of DNA damage, it detects DNA damage followed by repair and cell survival whereas at high levels of DNA damage, it activates the cell death pathways (Virag and Szabo, 2002). Upon DNA damage, PARP-1's zinc finger Zn1, Zn2 and Zn3 motifs have been reported to relay binding signals to catalytic domain followed by the recruitment of proteins involved in repair mechanism such as base excision repair [BER], single strand breaks [SSBs] and double strand breaks [DSBs] repair (D'Amours *et al*., 1999; Masutani *et al*., 2003). It also acts as a DNA damage sensor (Dantzer *et al*., 2000) and helps in chromatin

remodeling at DNA damage sites (Izhar *et al*., 2015). Several proteins like ALC1, histone macroH2A1.1, scaffold attachment factor SAFB1 are recruited to DNA damage sites via PARP-1 thus proving its indispensable role in DNA repair (Ahel *et al.,* 2009; Timinszky *et al*., 2009, Altmeyer *et al*., 2013). Evidences show the presence of PAR binding motifs in DNA damage response and checkpoint regulation proteins (Ahel *et al*., 2008; Rulten *et al*., 2008). PARP-2 has also shown to be involved in the later steps of BER/single-strand break repair (Mortusewicz *et al.*, 2007)**.** In nucleotide excision repair, PARP-1 inhibition or depletion has also shown to cause low efficiency of removal of UV-induced DNA damage (Robu *et al*., 2013). Among mammalian DNA repair pathways, PARP-1 has been also implicated in homologous recombination (Adamson *et al*., 2012) and non homologous end-joining pathways (Patel *et al*., 2011). PARP-1 has been reported to interact with replication fork protein (Timeless) in a PAR independent manner thereby allowing its recruitment to DSB sites to promote homologous recombination (Xie *et al*., 2015). Thus, it is clearly illustrated that PARP-1 plays a vital role in DNA damage response.

1.4.2 PARP-1 in Chromatin modulation

PARP-1 plays a key role in modulating the chromatin architecture by binding to the promoters of actively transcribed genes, thus regulating gene expression, especially in response to cellular signalling pathways (Krishnakumar *et al*. 2008; Frizzell *et al*. 2009). PARP-1 can directly modify the structural proteins that constitute the chromatin (D'Amours *et al.,* 1999). Histones form the main components of the chromatin assembly. Histones H1 and H2B show the most poly (ADP-ribosyl)ation *in vivo* and they are also the most preferred PARP-1 targets *in vitro* (Huletsky *et al.,* 1989; Poirier *et al.,* 1982), although all histones show modification to some extent (D'Amours *et al.,* 1999). PARylation of histones expose DNA, providing access to the protein machinery required for transcription and other processes. Nalabothula *et al*., (2015) sums up the possible mechanisms of chromatin structure remodeling by PARP-1 as: a) it binds between nucleosomes and linkers b) it PARylates histones, linker histones thus modifying

chromatin architecture c) it competes with histone H1 for nucleosome binding. All the above studies strengthen the role of PARP-1 in chromatin remodeling.

1.4.3 PARP-1 in transcription

Numerous *in vitro* and *in vivo* experiments have shown that PARP-1 behaves as chromatin modifier at the transcriptional level. Electrostatic repulsion between DNA and histones due to transfer of negatively charged PAR molecules onto acceptor proteins promotes transcription by recruiting transcriptional machinery (Pinnola *et al.,* 2007). PARP-1 is observed to be more localized at the promoter region of most actively transcribed genes (Krishnakumar *et al*., 2008). The transcriptional regulatory roles of PARP-1 are manifested mainly through two processes, modulating chromatin structure and acting as a part of enhancer/promoter binding complexes. Based on the cell type, it can enhance transcription with co-activators or inhibit transcription with repressors (Frizzell *et al*., 2009). Chromatin dependent gene expression is controlled by PARP-1, interacting with histones at promoter (Krishnakumar *et al*., 2008). Histone modifications like acetylation, phosphorylation or methylation are very essential for interaction between PARP-1 and DNA because it can add structural changes in histones (Berger, 2007). For e.g. phosphorylation of histone variant, H2Av promotes activity of PARP-1 in *Drosophila* at specific promoter region (Kotova *et al*., 2011). PARP-1 is also found to be localized at DNA repair site after binding to other histone variant, macroH2A (Kraus, 2009). Also, macroH2A1 stimulated H2B acetylation was seen in cancer progression which was PARP-1 dependent (Chen *et al*., 2014). Depletion of PARP-1 activity resulted into ineffective loading of RNA polymerase II transcriptional machinery implying its role in gene regulation (Krishnakumar and Kraus, 2010).

Reports also suggest that PARP-1 functions as a co-activator, which upregulates the transcription of nuclear factor erythroid 2 [NF-E2]-related factor 2 (Nrf2), promoting the interaction between Nrf2 and ARE (antioxidant response elements) (Wu *et al*., 2014). Reduced expression of connective tissue growth factor (CCN2) was found in tubular epithelial cells of kidney upon knockdown of PARP-1 (Okada *et al*., 2008). In addition to this, PARP-1 also functions as an insulator protein that organizes the genome into distinct

regulatory units by controlling the effects of enhancers on promoters, or by preventing the spread of heterochromatin (Ong *et al.,* 2013). *In vivo* and *in vitro* binding studies of PARP-1 and transcription factor Yin Yang 1 (YY1) suggested that PARP-1 plays a promoter regulatory role and inhibits the transcription of Cxcl12. In addition, changes in PARP-1- CCCTC-binding factor (CTCF) interactions due to serum shock induce the recruitment of circadian loci to the lamina leading to transcriptional attenuation (Zhao *et al*., 2015). PARP-1 is also known to be acting as an exchange factor thereby controlling transcription. Recently, it has been demonstrated that PARP-1 functions in the remodeling of promoter associated nucleosomes by replacing H2A.Z by H2A from *FOS* promoter to allow transcriptional activation in response to signaling by ERK (O'Donnell *et al.,* 2013).

The four proposed modes for modulation of gene expression by PARP-1 are shown in Fig.1. 5.

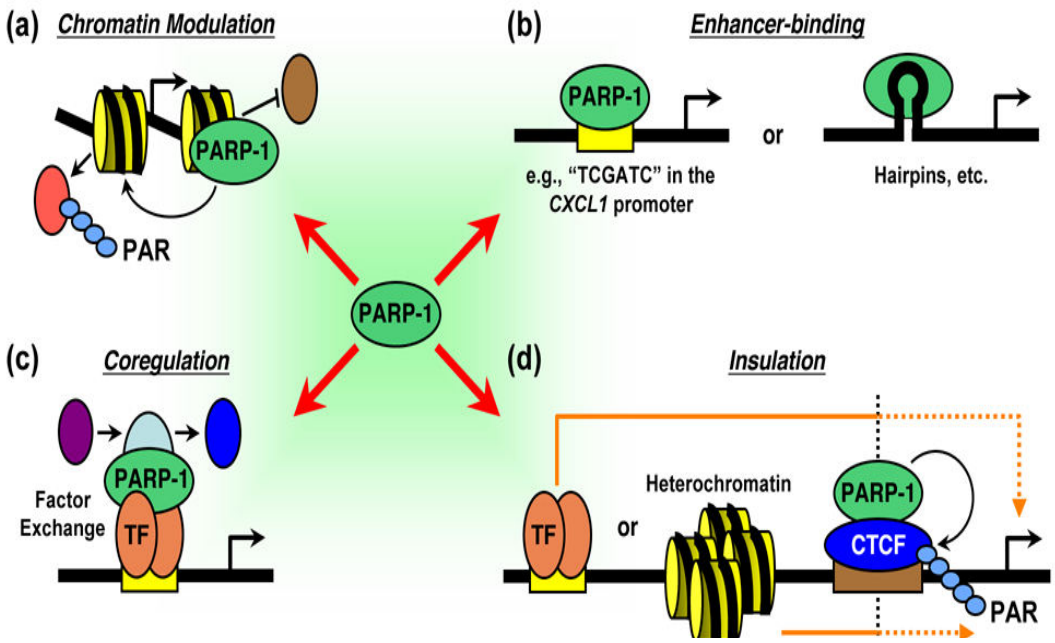

Fig. 1.5: Multiple modes of transcriptional regulation by PARP-1. PARP-1 regulates transcription in four ways, as indicated. **(a)** PARP-1 binds to nucleosomes thus modulating chromatin structure, by modifying histone proteins, or regulating the chromatin composition. **(b)** PARP-1 may bind to specific sequences or structures in the

DNA and thus acts as an enhancer-binding protein **(c)** PARP-1 may function as a promoter-specific "exchange factor" that stimulates the release and recruitment of inhibitory and stimulatory factors respectively in response to signal-regulated transcription. **(d)** PARP-1 can function as an insulator wherein PARylation prevents the relaxation of chromatin and binding of enhancers on promoters (Kraus, 2008).

The underlying mechanism of PARP-1 mediated transcriptional regulation is very complex and hence more studies are needed in this area to explore the transcriptional role of PARP-1.

1.4.4 PARP-1 in cellular-stress responses

Other than its role as a transcriptional regulator, PARP-1 is involved in cellular stress responses and cell death pathways. From various studies, it is evident that PARP-1 modulates the cellular stress responses through a series of processes that occur at multiple regulatory levels such as genomic, transcriptional, posttranscriptional, translational, and post-translational levels (Kim *et al.,* 2005) (Fig. 1.6).

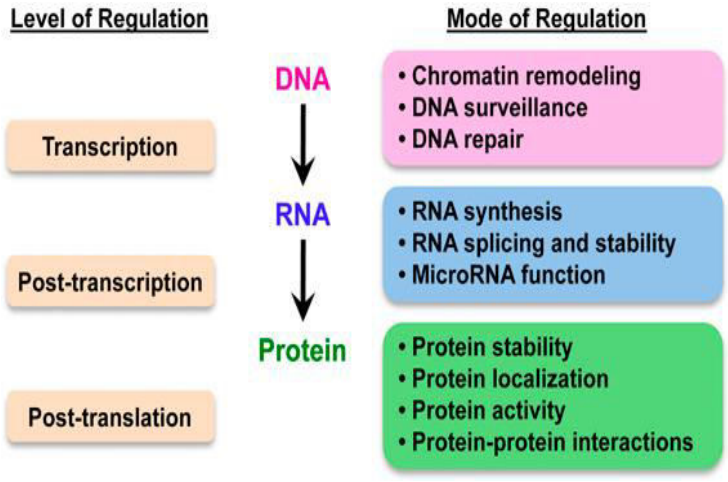

Fig. 1.6: PARP-1 modulates stress responses at multiple levels. (Luo and Kraus, 2012).

Depending on the type and the level of stress, PARP-1 generates different responses. The levels of PARP-1 activation decide the cell fate (*i.e.* cell survival or cell death). PARP-1 activation by mild or moderate stress leads to transcription and DNA repair mechanisms that aid to maintain genome stability and re-establish homeostasis (Kim *et al*., 2005; Gagne *et al*., 2006; Hassa and Hottiger 2008). In contrast, severe or sustained stress cause hyper-activation of PARP-1 resulting in the activation of distinct cell death programs, such as apoptosis or necrosis (Koh *et al*., 2005; David *et al*., 2009).

The PARP-1 activation mechanism that leads to cell death is still under active debate. A number of mechanisms have been proposed, including energy-failure-induced necrosis and apoptosis-inducing factor (AIF)-dependent apoptosis.

A. Apoptosis- Apoptosis is an ordered cell death process in which the cell is systematically dismantled within membrane-enclosed vesicles that are phagocytosed, thus preventing the release of intracellular components into the surrounding tissue (Edinger and Thompson, 2004).

B. Necrosis- Necrosis on the other hand, is cell death resulting from physical, chemical, or metabolic insults (e.g., the depletion of NAD^+ and ATP pools) (Koh *et al*. 2005). In contrast to apoptosis, during necrosis the cell swells and eventually ruptures, releasing cellular components into the surrounding tissue, which promotes an inflammatory response (Edinger and Thompson, 2004). In response to extensive DNA damage, over activation of PARP-1 leads to hypersynthesis of PAR which can promote cell death via necrosis, as a result of the depletion of cellular NAD^+ and ATP, and subsequent cellular energy failure (Decker and Muller, 2002; Bouchard *et al*., 2003).

C. Paraptosis- It has been described to be facilitated by mitogen activated protein kinases (MAPK) (Sperandio *et al*., 2004) and can be triggered by the TNF receptor family member TAJ/TROY (Wang *et al*., 2004), the insulin like growth factor I receptor (Sperandio *et al*., 2004) and PARP-1 via DNA damage. PARP-1 mediated paraptosis involves utilization of NAD^+ and depletion of energy levels, change in mitochondrial membrane potential, release of AIF and finally leading to cell death (Rajawat *et al*., 2014).

PARP-1 has been shown to play a role in the entire above said cell death programs (Koh *et al*., 2005; David *et al*. 2009). Yu *et al*., (2002) reported that PARP-1 also plays a role in caspase-independent apoptotic cell death through AIF. PARP-1 is also reported to be involved in DNA damage induced autophagy (Muñoz-Gámez *et al*., 2009). PARP-1 via autophagy displays a cytoprotective role in oxidative stress-induced necrotic cell death (Huang and Shen, 2009). Moreover, Son *et al*., (2011) have also reported that cadmium-mediated ROS generation leads to PARP-1 activation and energy (ATP) reduction, eventually culminating into autophagy in skin epidermal cells. PARP-1 is thus involved in various cell death pathways as shown in Fig. 1.7.

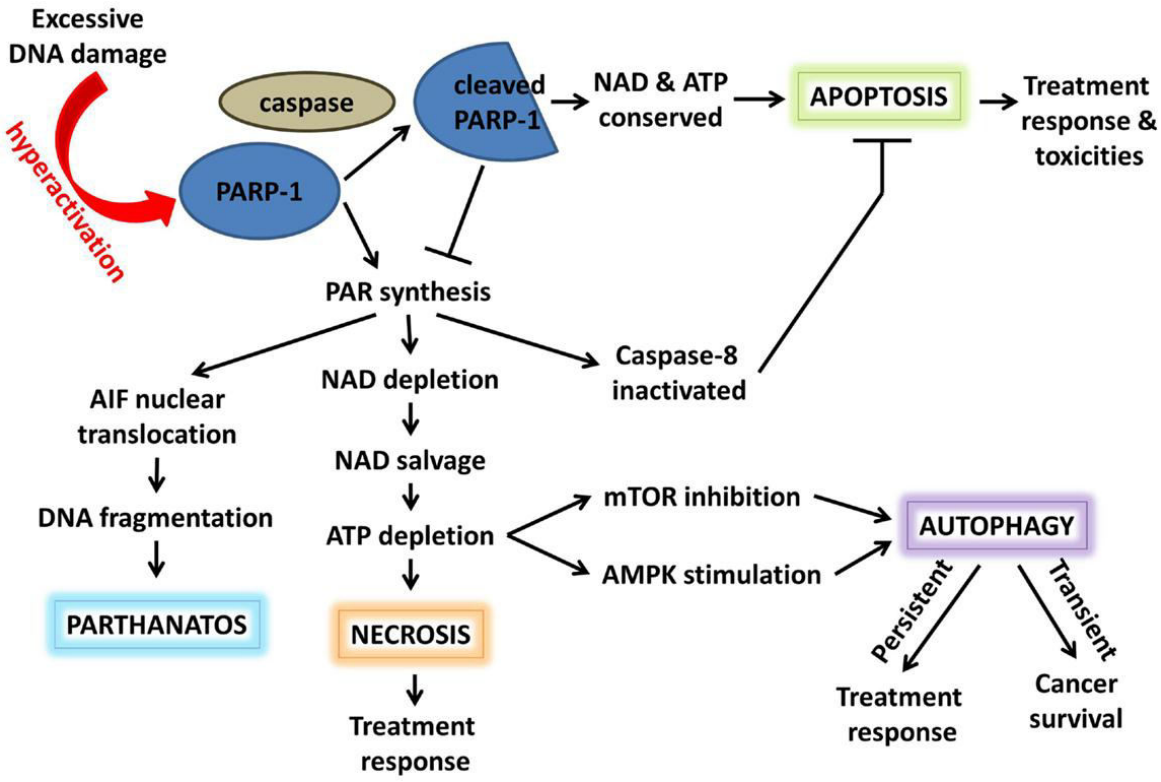

Fig. 1.7: PARP-1 mediated cell death. Over activation of PARP-1 and PAR synthesis leads to depletion of NAD^+ and consequently, ATP. Elevated PAR can promote necrosis, autophagy or AIF-induced PARP- mediated cell death. In addition, PARylation inactivates caspase-8, inhibiting apoptotic signaling. Alternatively, activated caspases can

cleave PARP-1 and the ensuing cleavage product inhibits uncleaved PARP-1, conserving NAD^+/ATP and promoting apoptosis. (Weaver and Yang, 2013)

Oxidative stress causes disruption in redox potentials that extend to the ER, causing accumulation of misfolded proteins, finally stimulating the Unfolded Protein Response (UPR) (Mansuri *et al*., 2014). It would be interesting to know if PARP-1 has a role in ER stress mediated cell death as it is upstream to autophagy, where PARP-1 is demonstrated to play an essential role. Hence, it is clear that PARP-1 is an essential regulator in many of the cell death pathways and this has been demonstrated in many tissues. However, a very interesting work by Jog and Carichio (2013) illustrates a characteristic difference in PARP-1 mediated necrosis in males and females. Male mice were shown to be prone to PARP-1 mediated necrosis while female mice showed PARP-1 independent cell death. Understanding the role of PARP-1 in different stress conditions and now even different sexes would help us to dissect out patho-mechanisms of various diseases.

1.4.5 PARP-1 and cancer

An error in the replication process, production of ROS and exposure to UV radiation result in DNA damage which includes single strand breaks [SSBs], double strand breaks [DSBs] etc. Cells then signal DNA repair pathways such as nucleic acid excision repair [NER], mismatch Repair [MMR], base excision repair [BER], non-homologous end joining [NHEJ] and homologous recombination [HR] resulting into cell survival with an exception of tumor cells. PARP -1 and PARP- 2 are key regulators for the function of DNA repair mechanisms however, in genetic disorders, such as BRCA1 and BRCA2 mutations, prevent DNA repair mechanism and increase the risk of malignancies (Hennessy *et al*., 2010). Inhibition of DNA repair process may lead to cell death and this brings PARP-1 as a perfect target for anticancer therapy. PARylation of targeted proteins by PARP-1 on an activation by SSBs and DSBs facilitates the recruitment of DNA repair proteins such as x-ray cross-complementing protein 1 (XRCC1) to DNA damage sites (El-Khamisy *et al*., 2003; Houtgraaf *et al*., 2006). PARP-1 may also facilitate homologous recombination (HR) through the recruitment of factors like Ataxia

telangiectasia- mutated (ATM), Nijmegen breakage syndrome 1 (Nbs1) and mitotic recombination 11 (Mre11) to sites of DSBs (Haince *et al.,* 2008). However, a major role in HR repair involves localization of BRCA-1 and BRCA-2. BRCA-1 plays an essential role in the surveillance of DNA damage and transduction of DNA repair responses while BRCA-2 is directly involved in double-stranded DNA repair, via modulation of Rad51 by HR (Tutt and Ashworth, 2002).

PARP-1 inhibition does not cause cell lethality by itself, since the cell has an intact HR pathway for DNA repair. Cells that have a mutated *BRCA1* or *BRCA2* genes like in cases of breast cancer or those that are deficient in BRCA1 or BRCA2 proteins like in the case of sporadic cancers, are found to be defective in their ability to repair DNA through HR and henceforth depend on error-prone Non-homologous end joining (NHEJ). This results in amplification of DNA instability and chromosomal aberrations eventually causing cell death (Fig. 1.8A). This synergistic effect has been very well demonstrated by Arun *et al*., (2015) wherein PARPi (PARP inhibitor) AZD2281 showed more promising results in BRCA1 and BRCA2 bearing mutants via induction of autophagy. This concept of synthetic lethality has been implemented upon in cancer therapeutics. In cases of breast and ovarian cancer, treatment with PARP-1 inhibitors, Olaparib and Veliparib (Approach 1), has shown positive clinical results (Fong *et al.,* 2010). Epigenetic modulation or artificial inactivation of BRCA pathway (Approach 2a) in cases of sporadic cancer along with the use of PARPi plays a key to therapeutics. This synergistic inhibition of DNA repair poses a double-hit mechanism for cancer cell death. PARPi can also be used in combination with chemotherapy and radiation (Approach 2b), rendering the cells prone to demise under enhanced damaged conditions. For example, in cases of non-Hodgkin lymphoma cell line, use of PARPi in combination with both external beam radiation and ^{131}I-tositumomab; radio sensitization with Veliparib in head and neck carcinoma cell lines and lung cancer xenograft models; or with Niraparib in neuroblastoma cell lines, and whole brain radiation in cases of brain metastases (Do and Chen, 2013) (Fig. 1.8B).

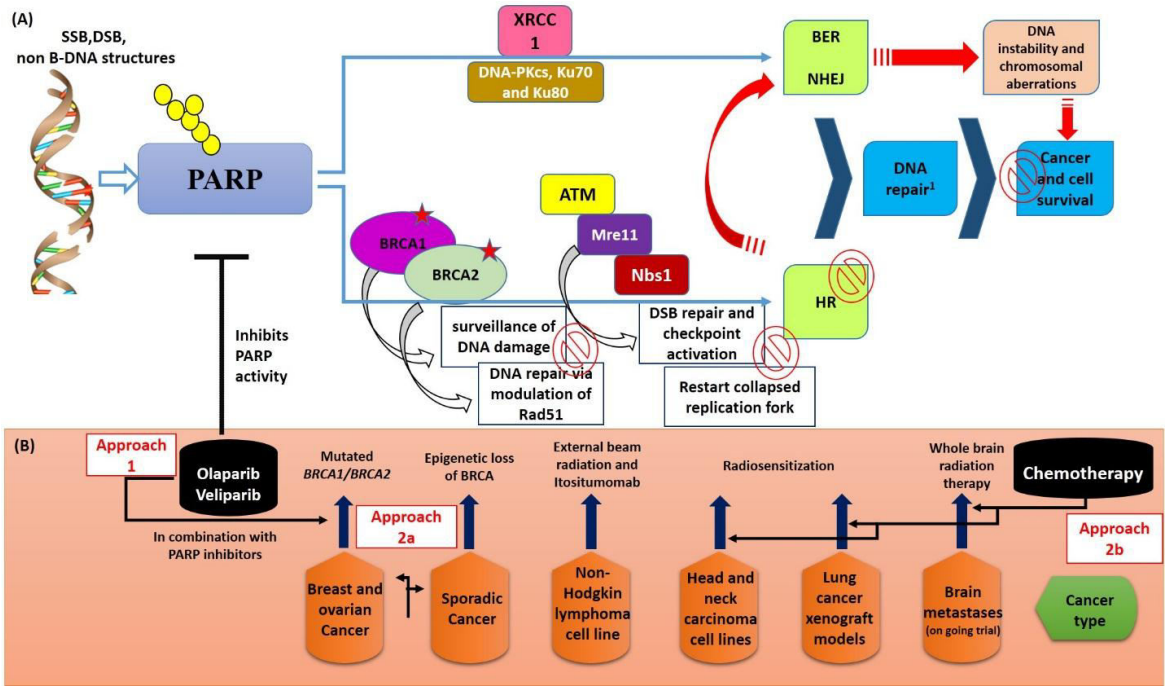

Fig. 1.8: PARP-1 and cancer therapy. (A) In normal cells, upon DNA damage like SSB, DSB, Non B-DNA structures, PARP-1 gets activated and thereby aids in the recruitment of DNA repair proteins such as the scaffolding protein XRCC1 to sites of SSBs through BER whereas DNA-PKcs, Ku70 and Ku80 to sites of DSBs through NHEJ. It also aids HR via recruitment of factors such as ATM, Nbs1 and Mre11 to sites of DSB's. Another very essential process of HR repair involves localization of BRCA-1 and BRCA-2 to sites of double stranded DNA damage. In cancer cells bearing BRCA1/2 mutations or deficiency (red star), cells are rendered fault in HR repair (red no symbol) and thus there is complete dependence on NHEJ (error prone) for DSB DNA repair and SSB for BER (red arrows); both of which are PARP-1 dependent. Thus, PARP inhibition serves as an excellent approach for therapy. BRCA1/2 mutations or deficiency along with PARP-1 inhibition leads to the amplification of DNA instability due to impairment in BER, NHEJ and ATM mediated HR repair and chromosomal aberrations results in cell death. (B) Approach1- PARP-1 inhibitors like Olaparib, Veliparib etc. have been promising therapeutic candidates in case of breast cancer and ovarian cancer. Approach 2a uses PARP-1 inhibitors in case of epigenetic modulation or artificial inactivation of BRCA pathway in case of sporadic cancers whereas approach 2b involves use of

chemotherapy and radiation along with PARP-1 inhibitor depending on the cancer type (Jubin *et al*., 2016b).

Thus, these studies suggest that the inhibition of PARP-1 has potential as a cancer therapeutant through at least two mechanisms: i] by potentiating chemotherapeutic agents that cause DNA damage and by increasing tumor sensitivity; and ii] by inducing "synthetic lethality" in cells that are highly dependent on PARP-1, due to deficiency in homologous recombination such as BRCA1 mutants.

1.4.6. PARP-1 in cell differentiation/multicellularity

Out of the various overlooked roles of PARP-1, its role in cell differentiation and multicellularity has been sparsely studied. However, accumulating reports in the model systems suggest a definite role of PARP-1 in growth and multicellularity. For example, Drosophila PARP has been shown to act in ectodermal specification and neural crest development in Zebrafish (Rouleau *et al*., 2011). PARP has been associated in development and cell differentiation from lower life forms to higher eukaryotes (Kawal *et al*., 2011). Our lab studies are indicative of PARP's role in *D. discoideum* development wherein its downregulation led to arrested development (Rajawat *et al*., 2011). Moreover, it is also reported that PARP may be essential in combating stress conditions in *Dictyostelium* (Rajawat *et al*., 2007; Couto *et al*., 2011; Rajawat *et al*., 2014 a, b; Mir *et al*., 2015). PARP-1 orthologs in fungus have also been demonstrated to result in defective development and decreased life span (Semighini *et al*., 2006; Muller-Ohldach *et al*., 2011). As we move to the higher life forms like plants, it was seen that AtPARP-1 and/or AtPARP2 knockdown altered *Arabidopsis* development (De Block *et al*., 2005) and AtPARP2 ortholog in oilseed rape [*Brassica napus*] did not affect its development (Vanderauwera *et al*., 2007). However, further work is needed to explore the role of PARP in plant development. In addition, studies in Drosophila also suggest importance of PARP in chromatin loosening at ecdysone inducible regions thereby inducing purparium formation and metamorphosis (Fletcher *et al*., 1995; Tulin and Spradling., 2003). These results are also substantiated by mice studies wherein PARP-1 and PARP-2 double mutant mice were found to be not viable and die at the onset of gastrulation, establishing the importance of both the PARPs during early embryogenesis (de Murcia *et al*., 2003).

Recently, Hamazaki *et al*., (2015) have shown that PARP inhibition caused inhibition of DNA demethylation of the *IL17d* promoter region at the 2-cell stage leading to downregulation of genes essential for early embryogenesis. Thus, it is clear from the above that PARP-1 is involved in differentiation and multicellularity, but the mechanisms by which PARP-1 controls differentiation are yet to be understood.

In view of the existing literature, PARP-1 appears to be a strong candidate involved in cell survival, cell death and differentiation. However, its role in cell growth and development remains elusive due to lack of in-depth studies. Hence, the focus of the present study is to understand the role of PARP-1 in cell growth and development. We have chosen the model system, *Dictyostelium discoideum*, a cellular slime mold to address this question. *D. discoideum* has pathways which are very similar to that found in higher complex organisms but unlike the yeast system, *D. discoideum* can form multicellular structures and differentiate into two cell types which allow the investigation of the role of PARP-1 in multicellularity as well as differentiation. Also, *Dictyostelium,* being a caspase independent system, proves to be an excellent model system for studying caspase independent cell death.

1.5 *Dictyostelium discoideum*

Dictyostelium discoideum is often referred to as 'Slime mold' or 'Social amoeba'. It is a haploid organism with 34Mb of DNA which is compacted into six chromosomes (Eichinger *et al*., 2005). This cellular slime mold exhibits a unique life cycle in the sense that it shows both unicellular as well as multicellular stages. Multicellular morphogenesis of this simple eukaryotic system makes it a preferred model for various cellular processes like development, differentiation, patterning etc. Also, the availability of sequenced *D. discoideum* genome along with extensive repertoire of well-developed biochemical and molecular techniques makes it an attractive model system. Genome sequencing of *D. discoideum* also revealed an interesting fact, that most of the genes show high degree of similarity with those of higher organisms. This is one of the organisms, having both unicellular and multicellular stages incorporated in its life cycle, thus correctly positioned at the crossroads between uni- and multicellular life, providing a link between the prokaryotes and the eukaryotes (Eichinger *et al*., 2005). *Dictyostelium* has many more

genes in common with animals than yeast species. Thus, *Dictyostelium* offers access to many protein classes that are not represented in the yeasts (Williams, 2010).

D. discoideum, in presence of adequate nutrient levels remains as undifferentiated, unicellular cells, capable of undergoing mitotic divisions at every 10-12 hrs. However, under nutrient starving conditions, the developmental phase of *D. discoideum* is induced. Cells become sensitive to cAMP gradients released from aggregation centers in a pulsatile manner. Approximately 100,000 cells converge to form aggregates. These developing structures are enveloped in extracellular sheath consisting of protein, cellulose and polysaccharides (Zhou-Chou *et al*., 1995; Freeze and Loomis, 1997a, b). As shown in the Fig. 1.9, after aggregation of the cells, a tip is formed which elongates into finger-shaped structure and subsequently develops into slug. This slug matures and culminates into mature fruiting body (Escalante *et al*., 2000).

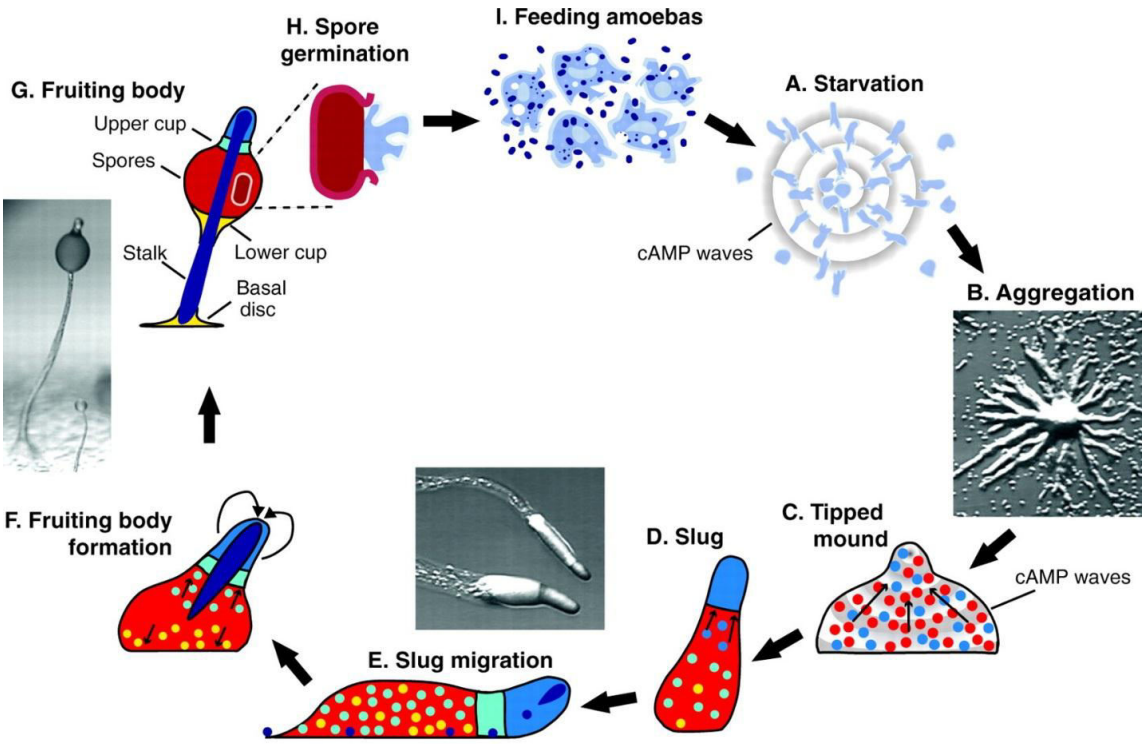

Fig. 1. 9: The life cycle of *Dictyostelium discoideum* Upon starvation, the amoebae enter a 4 to 8 hrs refractory period and amoebae within a certain area start migrating toward a single central amoeba, triggering a burst of gene activity in the amoebae. Possibly as many as 100 genes are called into action as new biochemical machinery is assembled: a set of glycoproteins that will enable cell–cell adhesions appear in the cell membrane. Next, adenylyl cyclase, an enzyme that catalyse the formation of cyclic AMP (cAMP; adenosine 3´, 5´-cyclic monophosphate) is formed from ATP; cAMP receptors are positioned in the cell membrane; and a membrane-bound phosphodiesterase that degrades cAMP appears as well as a phosphodiesterase inhibitor (Schaap, 2011).

In the case of *Dictyostelium* aggregation, intracellular cAMP levels first rise by the induction of adenylyl cyclase, and then cAMP is secreted out of the cell. The extracellular cAMP acts as a chemotactic agent, and the amoebae move in response to its presence, traveling up its concentration gradient. The cycle of cAMP secretion and amoeboid movement is repeated about once every 6 min. An amoeba pulses out cAMP and increases the cAMP gradient for about 60 sec, then remains stationary until the next pulse. Aggregation takes 8 to 12 hrs from the time of starvation. The assembly of amoebae first forms a mound or tight aggregate, which lengthens and finally tips over, becoming a finger-shaped slug. The slug, usually 2-4 mm long, can consist of only as many as 100,000 cells. It is motile having anterior and posterior ends, moves only forward, and it is attracted towards light, higher temperatures etc. that would attract it to the surface in its natural environment. Though, the slug may look relatively undifferentiated at first glance, it encompasses several types of cells. The anterior cells, constituting about 20% of the slug, are the prestalk cells which eventually form the stalk in the mature fruiting body. The posterior cells of the slug are primarily prespore cells, which become the spores of the fruiting body (Fig. 1.10). Apart from aggregation, cAMP continues to play a major role in differentiation too. High levels of cAMP appear to induce prespore cells, and also a morphogen called as DIF (differentiation-inducing factor), a low molecular weight lipid found in the anterior region of the slug, determines prestalk cells. This reorganization of prestalk cells and prespore cells takes approximately 8 to 10 hrs and results in a mature fruiting body, usually 1–2 mm high. The stalk cells

have now elevated the spore mass high above the substratum. It awaits the proper stimulus to release its spores to the substrate (Ashworth and Watts, 1970; Klein, 1988; Yu and Saxe, 1996; Mir *et al.,* 2007).

Pattern formation involving the precursors of the two terminally differentiated cell types can be clearly visualized during the slug stage where the anterior 10-15% is composed of prestalk cells while the rest 85-90% of the posterior region is made up of prespore cells. The prestalk cells and the prespore cells are the precursors of the terminally differentiated stalk (dead vacuolated) and spore (viable) cells, respectively.

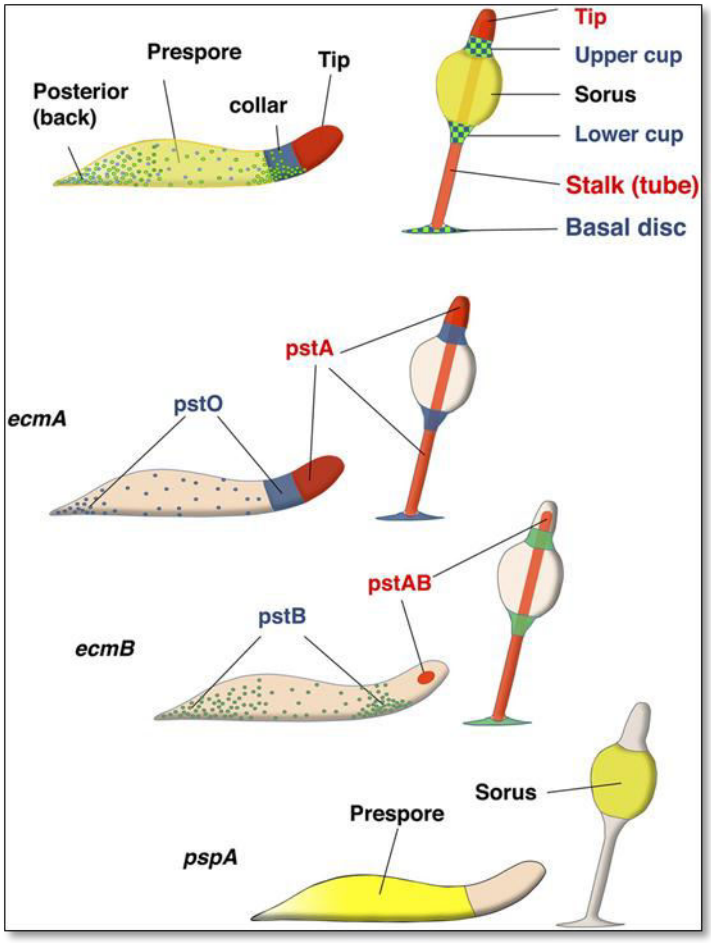

Fig. 1.10: Arrangement of cell types in *Dictyostelium* slug and culminants Left panel depicts parts of *Dictyostelium* slug while the fruiting body is shown at the right. Prespore cells are shown in yellow eventually form sorus and red cells denoting pstA or AB form

stalk cells. pstO (blue) and pstB (green) cells are the DIF dependent cell types which eventually form upper/lower cup and basal disc respectively (Chattwood *et al*., 2013).

1.5.1 Genes/ Factors responsible for the transition from growth to development

1.5.1.1 Pre Starvation Factor (PSF):

Throughout the vegetative growth, *Dictyostelium discoideum* amoebae secrete an autocrine factor known as PSF. It is a 68kDa glycoprotein that is secreted while cells are in growth phase & accumulates as an indicator of the ratio of the cell density to food supply. PSF induces genes such as discoidin-I & cyclic nucleotide phosphodiesterase (PDE) that activate the developmental morphogenesis (Ashworth and Watts, 1970; Mir *et al*., 2007).

1.5.1.2 Conditioned Medium Factor (CMF):

CMF is an 80 kDa glycoprotein sequestered in vegetative cells and secreted upon starvation regardless of cell density. CMF increases the frequency of pseudopodia formation & hence is important for chemotaxis (Mir *et al*., 2007).

1.5.1.3 Myb2:

Dictyostelium discoideum Myb2 is a transcription factor that contains 3 Myb repeats, a DNA-binding helix-turn-helix motif, a potential nuclear localization signal, and a guanine-proline and acidic amino acids-rich regions of Myb-related transcription factor. This Myb2 mediates the initial induction of adenylyl cyclase plays a central role in the transition from growth to development in *Dictyostelium discoideum* (Mir *et al*., 2007).

1.5.1.4 DIAI:

Cells in any phase of the cell cycle can start differentiating. Several genes are expressed in response to initial differentiation. One such gene is *DIAI*, which is expressed at 2 hrs after starvation, reaching a peak at 4 hrs followed by a rapid decrease in its levels. Expression of *DIAI* is transient and it is seen only during initial stages of development.

DIAI over expression impedes differentiation and such cells showed aggregation defects (Mir *et al*., 2007).

1.5.1.5 Cyclic AMP (cAMP):

In the social amoeba *Dictyostelium discoideum*, cAMP via PKA controls almost all the major life cycle transitions including growth to development. Aggregation of amoebae into multicellular structures is organized by cAMP, which acts as a chemo-attractant and a morphogen. Once cAMP reaches the threshold point, it triggers the cascade of events (Mir *et al*., 2007).

1.5.1.6 cAMP receptor 1(CAR1):

It is a G-protein coupled receptor that controls the chemotactic cell movement during *D. discoideum* development. It facilitates two distinct responses in nutrition deprived amoebae: i) produces and relays the cAMP signal; ii) responds to cAMP (i.e. chemotaxis) (Konijn *et al*., 1969; Roos *et al*., 1975; Shaffer, 1975). cAMP is secreted by each cell during aggregation which interacts with high affinity cAMP receptors (cAR) like cAR1 to mediate chemotaxis (Saxe *et al*., 1993). Four developmentally regulated cAR receptors namely cAR1, cAR2, cAR3 and cAR4 are present. The cAR2 and cAR4 receptors are expressed during the late developmental stages and show lower affinity for cAMP (Louis *et al*., 1994; Saxe *et al*., 1996) while cAR3 is expressed simultaneously with cAR1, but it has decreased affinity to cAMP than cAR1 (Johnson *et al*., 1993). cARs bind to extracellular cAMP leading to activation of adenylyl cyclase thus producing more cAMP (Roos *et al*., 1975; Tomchik and Devreotes, 1981). Endogenous cAMP can induce spore differentiation (Barklis and Lodish, 1983; Mehdy *et al*., 1983) as well as stalk cell differentiation (Bretschneider *et al*., 1995).

1.5.1.7 YAKA:

It is a cytosolic protein kinase which phosphorylates itself. It is one such factor that facilitates the exit from the cell cycle and mediates transition from growth to development. Induction of *YAKA* upon starvation leads to a decrease in gene expression expressed during vegetative phase such as *CPRD* and induces the expression of *PKA,*

ACA and *CAR1*. *YAKA* is expressed at basal level during growth phase, and peaked at the onset of starvation and then decreases but is present throughout the development (Klien, 1998; Mir *et al*., 2007).

1.5.1.8 Adenylate cyclase A (ACA):

ACA, a G-protein-coupled adenylate cyclase is one of the first genes to be expressed upon starvation. It produces extracellular cAMP, which is the signaling molecule required for chemotaxis and aggregation of neighboring cells. Genes encoding three distinct adenylyl cyclases have been characterized with expression at different stages of *D. discoideum* (Wessels *et al*., 2000; Mir *et al*., 2007). ACA is involved in cAMP production during initial aggregation processes (Rodrigues and Sastre, 2016).

1.5.1.9 Protein kinase (PKA):

PKA, a cAMP dependent protein kinase plays multiple roles during *Dictyostelium discoideum* development & it is the central component in signal transduction pathway. Inactive form of PKA consists of catalytic subunit (PKA-C) associated with regulatory subunit (PKA-R). Exponentially growing *Dictyostelium* cells consists of both subunits as PKA is not required for growth, there is a 5-fold increase in the catalytic subunit in the first 6 hrs of the development & is maintained till culmination. The signal transduction pathway that initiates from binding of cAMP on cell surfaces to accumulation of transcripts of developmental genes appears to act through PKA (Mir *et al*., 2007).

1.5.1.10 Counting factor (CF):

Individual cell would be able to sense the number of cells in a group by secreting and sensing a diffusible factor known as Counting Factor, which is required for the proper function of cell counting mechanism that regulates organism size. CF is a large complex of >450kDa of at least 5 polypeptides with 60, 50, 45, 40, 30 kDa, and it's over secretion leads to the formation of smaller fruiting bodies. A high extracellular concentration of CF indicates that there is a large number of cells which then causes the aggregation stream to break up. CF does indeed increase cell-cell adhesion as well as cell motility (Mir *et al*., 2007).

1.5.1.11 RegA:

It is an intracellular phosphodiesterase. Its activity removes the signal by intracellular hydrolysis of the cAMP. Previous studies suggest that *RegA*$^-$ cells formed small aggregates and did not form stream indicates that this gene product is required for the directional movement during streaming stage in order to move towards the aggregation center and aggregate further (Mir *et al*., 2007; Wessels *et al*., 2000).

1.5.1.12 CsA:

It is a cell adhesion molecule. This glycoprotein starts accumulating in a pre-aggregation stage and is maximally expressed during aggregation and disappears after aggregate compaction. *CsA* gene disruption dramatically affects the ability of cells to aggregate & form fruiting bodies with viable spores, while constitutive expression of *CsA* under actin promoter leads to early aggregation & fruiting body formation (Ponte *et al*., 1998).

1.5.1.13 PdsA:

PdsA is an extracellular phosphodiesterase enzyme. In order to maintain required levels of extracellular cAMP, this enzyme cleaves cAMP molecules as the levels are raised. Reports suggest that alteration of *PdsA* gene expression leads to aggregation defects in the *D. discoideum* which indicates its importance in the process of starvation induced development (Mann *et al*., 1989).

The aggregation network in *Dictyostelium* is summarized in Fig.11

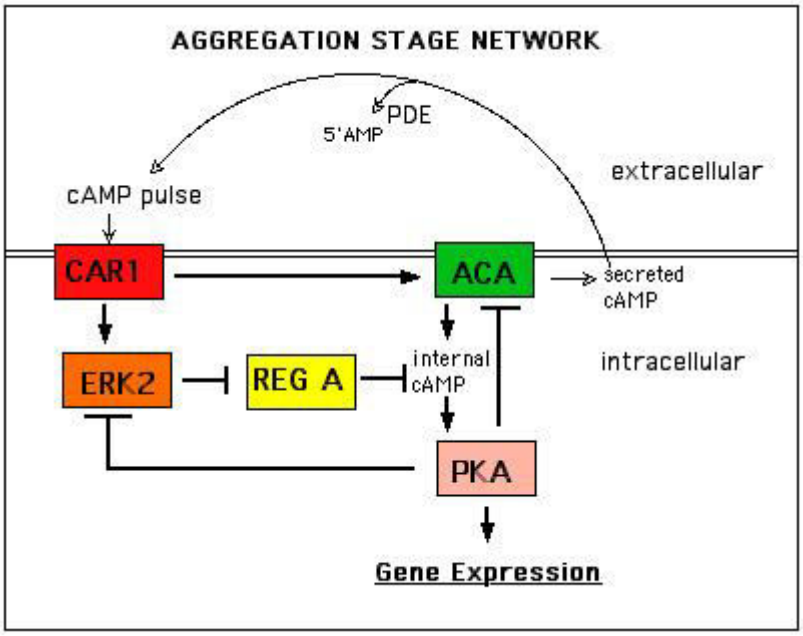

Fig. 1.11: ***Dictyostelium discoideum*** **cAMP signalling system.** Secreted cAMP binds to CAR1 thus activating ACA leading to further production of cAMP. Intracellular cAMP leads to activation of PKA eventually leading to developmental gene expression. Extracellular cAMP is controlled by phosphodiesterases like PDE, PDSA. Intracellular cAMP is regulated by phosphodiesterase REGA which is controlled by ERK2 (Laub and Loomis, 1998).

1.6 Other Factors controlling differentiation

The mechanism of pattern formation in this organism suggests that both positional information and predetermination and sorting out of cells play significant role (Nanjundiah and Saran, 1992).

Saran *et al*., (1994) have shown that cells which are larger in size tend to become stalk cells while the smaller cells become spore cells. The spore cells have lower intracellular calcium levels compared to the stalk cells. Maeda *et al.,* (1989) have shown that an increase in calcium levels favors prestalk pathway. Cells in S and early G2 phases have relatively high levels of cellular Ca^{2+} and exhibit a prestalk tendency after starvation, whereas cells in mid to late G2 (or with a high DNA content) have relatively low levels of Ca^{2+} and display a prespore tendency (Azhar *et al*., 2001). Leach *et al*., (1973) have shown that cells grown in presence of high glucose when mixed with cells grown in

presence of low glucose, the former tend to form spore cells while the latter form stalk cells.

Another significant factor in determining cell fate is ammonia which is a byproduct of protein degradation occurring during development (Schindler and Sussman, 1977; Wilson and Rutherford, 1978; Fong and Bonner, 1979). It acts as an inhibitor of DIF, which is essential for stalk cell differentiation and thus it blocks the fruiting body formation (Wang and Schaap, 1989). Depletion of ammonia increases the proportion of prestalk cells (Feit *et al*., 1990). An increase in ammonia concentration was also found to interfere with the cAMP signaling and its relay mechanism (Williams *et al*., 1984).

1.7 *Dictyostelium discoideum*: a model system

1.7.1 Cell Differentiation

Developmental morphogenesis in *D. discoideum* involves complex network of gene regulatory mechanisms and coordinated cell type differentiation leading to a final fruiting body. The availability of biochemical and molecular techniques and sequenced genome has enabled the discovery of complex signaling networks involved in *D. discoideum* development and differentiation which can be further extrapolated to higher eukaryotes. Developmental and cell type specific gene expression and differentiation have been extensively characterized in this model which can be implicated in higher eukaryotes.

1.7.2 Chemotaxis

Chemotaxis is the directional movement of a cell in response to a chemical gradient. Many cell types exhibit this phenomenon like, bacteria and amoebae can move in the direction of food source. Immune cells like macrophages and neutrophils can move towards an invading cell. The overall architecture of the networks, as well as the individual signaling components, is highly conserved between *Dictyostelium* and mammalian leukocytes. Hence, *D. discoideum* proves to be an excellent system for the study of chemotaxis wherein proteins involved in these processes can be identified to pin point new targets in higher complex organisms (Aubry and Firtel, 1999).

1.7.3 Signal transduction

A detailed study of signaling system in *D. discoideum* provides indications to the mechanisms of intercellular communication during development of metazoans. There are numerous signaling pathways involved in chemotaxis, cell-cell interaction, cell proportioning and differentiation. Due to conserved behavior of many proteins and mechanisms in higher organisms, it is not surprising that *D. discoideum* could lead to the finding of molecules involved in cell signaling pathways (Aubry and Firtel, 1999).

1.7.4 Phagocytosis

D. discoideum helped in proposing a model for recognition in lectin type receptor and non receptor mediated phagocytosis (Vogel *et al*., 1980). *D. discoideum* is a popular model system for studying phagocytosis as many of the similar intracellular signaling molecules and regulators do appear in mammalian system (Bush *et al*., 1996; Harris *et al*., 2001).

1.7.5 Cell Death

D. discoideum exhibits caspase independent type of cell death (paraptosis) during its development which occurs even in the presence of caspase inhibitors (Olie *et al.,* 1998) Paraptosis is characterized by the absence of oligonucleosomal DNA fragmentation. It also involves mitochondrial membrane potential (Ψm) loss leading to the release of AIF from the mitochondria (Arnoult *et al*., 2001). Blast search results suggest that *D. discoideum* has a paracaspase gene, neither a metacasapase nor a caspase gene. As *D. discoideum* shares similarity with cell death in mammalian cells and thus it offers a good model system for characterization of paraptotic cell death. Thus the social amoeba *D. discoideum*, a powerful paradigm provides clear insight into the regulation of growth and development.

In view of the above discussion, *D. discoideum* is an excellent model system to study the role of PARP in growth and multicellularity.

1.8 References

1. Adamson, B., Smogorzewska, A., Sigoillot, F.D., King, R.W., Elledge, S.J., 2012. A genome-wide homologous recombination screen identifies the RNA-binding protein RBMX as a component of the DNA-damage response. Nat. Cell Biol. 14, 318-328.
2. Ahel, D., Hořejší, Z., Wiechens, N., Polo, S.E., Garcia-Wilson, E., Ahel, I., *et al*., 2009. Poly (ADP-ribose) dependent regulation of DNA repair by the chromatin remodeling enzyme ALC1. Science. 325, 1240-1243.
3. Ahel, I., Ahel, D., Matsusaka, T., Clark, A.J., Pines, J., Boulton, S. J., *et al*., 2008. Poly (ADP-ribose)-binding zinc finger motifs in DNA repair/checkpoint proteins. Nature. 451, 81-85.
4. Altmeyer, M., Toledo, L., Gudjonsson, T., Grøfte, M., Rask, M.B., Lukas, C., *et al*., 2013. The chromatin scaffold protein SAFB1 renders chromatin permissive for DNA damage signaling. Molecular Cell. 52, 206-220.
5. Alvarez-Gonzalez, R., Spring, H., Müller, M., & Bürkle, A., 1999. Selective loss of poly (ADP-ribose) and the 85-kDa fragment of poly (ADP-ribose) polymerase in nucleoli during alkylation-induced apoptosis of HeLa cells. Journal of Biological Chemistry. 274, 32122-32126.
6. Ame, J.C., Spenlehauer, C., de Murcia, G., 2004. The PARP superfamily. Bioessays. 26, 882-893.
7. Arun, B., Akar, U., Gutierrez-Barrera, A.M., Hortobagyi, G.N., Ozpolat, B., 2015. The PARP inhibitor AZD2281 (Olaparib) induces autophagy/mitophagy in BRCA1 and BRCA2 mutant breast cancer cells. Int. J. Oncol. 47, 262-268.
8. Berger, S.L., 2007. Thecomplex language of chromatin regulation during transcription. Nature. 447, 407-412.
9. Bouchard, V.J., Rouleau, M., Poirier, G.G., 2003. PARP-1, a determinant of cell survival in response to DNA damage. Exp. Hematol. 31, 446–454.
10. Burkle A., Laszlo V., 2013. Poly (ADP-ribose): PARadigms and PARadoxes. Journal of Molecular Aspects of Meidicine. 34, 1046-1065.

11. Chen, H., Ruiz, P.D., Novikov, L., Casill, A.D., Park, J.W., Gamble, M.J., 2014. MacroH2A1.1 and PARP-1 cooperate to regulate transcription by promoting CBP-mediated H2B acetylation. Nat. Struct. Mol. Biol. 21, 981-989.
12. Cohen-Armon, M., 2007. PARP-1 activation in the ERK signaling pathway. Trends Pharmacol. Sci. 28, 556-560.
13. Cohen-Armon, M., Visochek, L., Rozensal, D., Kalal, A., Geistrikh, I., Klein, R., *et al*., 2007. DNA-independent PARP-1 activation by phosphorylated ERK2 increases Elk1 activity: a link to histone acetylation. Mol. Cell. 25, 297-308.
14. D'Amours, D., Desnoyers, S., D'Silva, I., Poirier, G.G., 1999. Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. Biochem. J. 342, 249-268.
15. Dantzer, F., de La Rubia, G., Menissier-De, M.J., Hostomsky, Z., de Murcia, G., Schreiber, V., 2000. Base excision repair is impaired in mammalian cells lacking poly(ADP-ribose) polymerase-1. Biochemistry. 39, 7559-7569.
16. David, K. K., Andrabi, S. A., Dawson, T. M., Dawson, V. L., 2009. Parthanatos, a messenger of death. Frontiers in bioscience (Landmark edition). 14, 1116.
17. Davidovic, L., Vodenicharov, M., Affar, E. B., Poirier, G. G., 2001. Importance of poly (ADP-ribose) glycohydrolase in the control of poly (ADP-ribose) metabolism. Experimental cell research. 268, 7-13.
18. Decke, P., Muller, S., 2002. Modulating poly (ADP-ribose) polymerase activity: Potential for the prevention and therapy of pathogenic situations involving DNA damage andoxidative stress. Curr. Pharm. Biotechnol. 3, 275–283.
19. Dong, F., Soubeyrand, S., Haché, R.J., 2010. Activation of PARP-1 in response to bleomycin depends on the Ku antigen and protein phosphatase 5. Oncogene. 29, 2093-2103.
20. Dunn, C.A., O'Handley, S.F., Frick, D.N., Bessman, M.J., 1999. Studies on the ADP-ribose pyrophosphatase subfamily of the nudix hydrolases and tentative identification of trgB, a gene associated with tellurite resistance. J. Biol. Chem. 274, 32318–32324.
21. Edinger, A.L.,Thompson, C.B., 2004. Death by design: Apoptosis, necrosis and autophagy. Curr. Opin. Cell Biol. 16, 663–669.

22. El-Khamisy, S.F., Masutani, M., Suzuki, H., Caldecott, K.W., 2003. A requirement for PARP-1 for the assembly or stability of XRCC1 nuclear foci at sites of oxidative DNA damage. Nucleic Acids Res. 31, 5526-5533.
23. Eustermann, S., Videler, H., Yang, J.C., Cole, P.T., Gruszka, D., Veprintsev, D., *et al*., 2011. The DNA-binding domain of human PARP-1 interacts with DNA single-strand breaks as a monomer through its second zinc finger. J. Mol. Biol. 407, 149-170.
24. Eustermann, S., Wu, W.F., L, M.F., Yang, J.C., Easton, L.E., Riccio, A.A., *et al.,* 2015. Structural Basis of Detection and Signaling of DNA Single-Strand Breaks by Human PARP-1. Mol. Cell. 60, 742-754.
25. Fong, P.C., Yap, T.A., Boss, D.S., Carden, C.P., Mergui-Roelvink, M., Gourley, C., *et al*., 2010. Poly(ADP-ribose) polymerase inhibition: frequent durable responses in BRCA carrier ovarian cancer correlating with platinum-free interval. J. Clin. Oncol. 28, 2512-2519.
26. Frizzell, K.M., Gamble, M.J., Berrocal, J.G., Zhang, T., Krishnakumar, R., Cen, Y., 2009. Global analysis of transcriptional regulation by poly (ADP-ribose) polymerase-1 and poly (ADP-ribose) glycohydrolase in MCF-7 human breast cancer cells. J. Biol. Chem. 284, 33926-33938.
27. Gagné, J. P., Hendzel, M. J., Droit, A., Poirier, G. G. 2006. The expanding role of poly (ADP-ribose) metabolism: current challenges and new perspectives. Curr. Opin. Cell Biol. 18, 145-151.
28. Haince, J.F., McDonald, D., Rodrigue, A., Dery, U., Masson, J.Y., Hendzel, M.J., *et al*., 2008. PARP-1 dependent kinetics of recruitment of MRE11 and NBS1 proteins to multiple DNA damage sites. J. Biol. Chem. 283, 1197-1208.
29. Hassa, P. O., Hottiger, M. O., 2002. The functional role of poly (ADP-ribose) polymerase 1 as novel coactivator of NF-κB in inflammatory disorders. Cell. Mol. Life Sci. CMLS. 59, 1534-1553.
30. Hassa, P.O., Haenni, S.S., Elser, M., Hottiger, M.O., 2006. Nuclear ADP-ribosylation reactions in mammalian cells: where are we today and where are we going? Microbiol. Mol. Biol. Rev. 70, 789-829.

31. Hassa, P.O., Hottiger M.O., 2008. The diverse biological roles of mammalian PARPS, a small but powerful family of poly-ADP-ribose polymerases. Front Biosci. 13, 3046–3082.

32. Hennessy, B.T., Timms, K.M., Carey, M.S., Gutin, A., Meyer, L.A., Flake, D.D. 2nd, 2010. Somatic mutations in BRCA1 and BRCA2 could expand the number of patients that benefit from poly(ADP-ribose) polymerase inhibitors in ovarian cancer. J. Clin. Oncol. 28, 3570-3576.

33. Hottiger, M.O., Hassa, P.O., Luscher, B., Schuler, H., Koch-Nolte, F., 2010. Toward a unified nomenclature for mammalian ADP-ribosyltransferases. Trends Biochem. Sci. 35, 208-219.

34. Houtgraaf, J.H., Versmissen, J., van der Giessen, W.J., 2006. A concise review of DNA damage checkpoints and repair in mammalian cells. Cardiovasc. Revasc. Med. 7, 165-172.

35. Huang, Q., Shen, H.M., 2009. To die or to live: the dual role of poly(ADP-ribose) polymerase-1 in autophagy and necrosis under oxidative stress and DNA damage. Autophagy. 5, 273-276.

36. Huletsky, A., de Murcia, G., Muller, S., Hengartner, M., Menard, L., Lamarre, D., *et al.*, 1989. The effect of poly(ADP- ribosyl)ation on native and H1-depleted chromatin. A role of poly(ADP-ribosyl)ation on core nucleosome structure. J. Biol. Chem. 264, 8878-8886.

37. Izhar, L., Adamson, B., Ciccia, A., Lewis, J., Pontano-Vaites, L., Leng, Y., *et al.*, 2015. A systematic analysis of factors localized to damaged chromatin reveals PARP-dependent recruitment of transcription factors. Cell Rep. 11, 1486-1500.

38. Jog, N.R., Caricchio R., 2013. Differential regulation of cell death programs in males and females by Poly (ADP-Ribose) Polymerase-1 and 17β estradiol. Cell Death Dis. 4, e758.

39. Ju, B., Solum, D., Song, E.J., Lee, K., Rose, D.W., Glass, C.K., *et al.*, 2004. Activating the PARP-1 sensor component of the Groucho/TLE1 corepressor complex mediates a CaMKinase IIdelta-dependent neurogenic gene activation pathway. Cell. 119, 815-829.

40. Jubin, T., Kadam, A., Jariwala, M., Bhatt S., Sutariya S., Satyendra G., *et al*., 2016b. Insights into the functional aspects of Poly [ADP-ribose] polymerase in cellular growth and differentiation. Cell Prol. (In Press)
41. Kameshita, I., Matsuda, Z., Taniguchi, T., Shizuta, Y., 1984. Poly(ADP-ribose) synthetase. Separation and identification of three proteolytic fragments as the substrate-binding domain, the DNA-binding domain and the automodification domain. J. Biol. Chem. 259, 4770-4776.
42. Kauppinen, T.M., Chan, W.Y., Suh, S.W., Wiggins, A.K., Huang, E.J., Swanson, R.A., 2006. Direct phosphorylation and regulation of poly(ADP-ribose) polymerase-1 by extracellular signal regulated kinases 1/2. Proc. Natl. Acad. Sci. USA. 103, 7136-7141.
43. Kickhoefer, V.A., Siva, A.C., Kedersha, N.L., Inman, E.M., Ruland, C., Streuli, M., *et al*., 1999. The 193-kD vault protein, VPARP, is a novel poly(ADP-ribose) polymerase J. Cell. Biol. 146, 917-928.
44. Kim, M.Y., Zhang, T., Kraus, W.L., 2005. Poly(ADP-ribosyl)ation by PARP-1: 'PARlaying' NAD^+ into a nuclear signal. Genes Dev. 19, 1951-1967
45. Ko, H.L., Ren, E.C., 2011. Novel poly(ADP-ribose) polymerase 1 binding motif in hepatitis B virus core promoter impairs DNA damage repair. Hepatology. 54, 1190-1198.
46. Koh, D.W., Dawson, T.M., Dawson, V.L., 2005. Mediation of cell death by poly(ADP-ribose) polymerase-1. Pharmacol Res. 52, 5–14.
47. Kotova, E., Lodhi, N., Jarnik, M., Pinnola, A.D., Ji, Y., Tulin, A.V., 2011. Drosophila histone H2A variant (H2Av) controls poly (ADP-ribose) polymerase1 (PARP1) activation in chromatin. Proc. Nat. Acad. Sci.108, 6205-6210.
48. Kraus, W. L., 2009. New functions for an ancient domain. Nat. Struct. Mol. Biol. 16, 904 - 907.
49. Kraus, W. L., Lis, J. T., 2003. PARP goes transcription. Cell. 113, 677-683.
50. Kraus, W., 2008 Transcriptional control by PARP-1: chromatin modulation, enhancer-binding, coregulation, and insulation. Current Opinion in Cell Biology. 20, 294–302

51. Krishnakumar, R., Gamble, M.J., Frizzell, K.M., Berrocal, J.G., Kininis, M., Kraus, W.L., 2008. Reciprocal binding of PARP-1 and histone H1 at promoters specifies transcriptional outcomes. Science. 319, 819-821.
52. Krishnakumar, R., Kraus, W.L., 2010. PARP-1 regulates chromatin structure and transcription through a KDM5B-dependent pathway. Mol. Cell. 39, 736-749.
53. Krishnakumar, R., Kraus, W.L., 2010. PARP-1 regulates chromatin structure and transcription through a KDM5B-dependent pathway. Mol. Cell 39, 736-749.
54. Kun, E., Kirsten, E., Ordahl, C.P., 2002. Coenzymatic activity of randomly broken or intact double-stranded DNAs in auto and histone H1 trans-poly(ADP-ribosylation), catalyzed by poly(ADP-ribose) polymerase (PARP I). J. Biol. Chem. 277, 39066-39069.
55. Langelier, M.F., Planck, J.L., Roy, S., Pascal, J.M., 2011. Crystal structures of poly (ADP-ribose) polymerase-1 (PARP-1) zinc fingers bound to DNA structural and functional insights into DNA-dependent PARP-1 activity. J. Biol. Chem. 286, 10690-10701.
56. Langelier, M.F., Planck, J.L., Roy, S., Pascal, J.M., 2012. Structural basis for DNA damage–dependent poly (ADP-ribosyl)ation by human PARP-1. Science. 336, 728-732.
57. Langelier, M.F., Ruhl, D.D., Planck, J.L., Kraus, W.L., Pascal, J.M., 2010. The Zn3 domain of human poly(ADP-ribose) polymerase-1 (PARP-1) functions in both DNA-dependent poly(ADP-ribose) synthesis activity and chromatin compaction. J. Biol. Chem. 285, 18877-18887.
58. Langelier, M.F., Servent, K.M., Rogers, E.E., Pascal, J.M., 2008. A third zinc-binding domain of human poly(ADP-ribose) polymerase-1 coordinates DNA dependent enzyme activation. J. Biol. Chem. 283, 4105-4114.
59. Lautier, D., Lagueaux, J., Thibodeau, J., Ménard, L., Poirier, G.G., 1993. Molecular and biochemical features of poly (ADP-ribose) metabolism. Mol. Cell Biochem. 122, 171-193.
60. Lonskaya, I., Potaman, V.N., Shlyakhtenko, L.S., Oussatcheva, E.A., Lyubchenko, Y.L., Soldatenkov, V.A., 2005. Regulation of poly(ADP-ribose)

polymerase-1 by DNA structure-specific binding. J. Biol. Chem. 280, 17076-17083.

61. Loseva, O., Jemth, A.S., Bryant, H.E., Schüler, H., Lehtiö, L., Karlberg, T., *et al*., 2010. PARP-3 is a mono-ADP-ribosylase that activates PARP-1 in the absence of DNA. J. Biol. Chem. 285, 8054-8060.
62. Luo, X., Kraus, W. L. 2012. On PAR with PARP: cellular stress signaling through poly (ADP-ribose) and PARP-1. Genes & development. 26, 417-432.
63. M.Y. Kim., S. Mauro., N. Gévry., J.T. Lis., W.L. Kraus., 2004. NAD+-dependent modulation of chromatin structure and transcription by nucleosome binding properties of PARP-1, Cell. 119, 803-814.
64. Ma, Q., Baldwin, K.T., Renzelli, A.J., McDaniel, A., Dong, L., 2001. TCDD-inducible poly(ADP-ribose) polymerase: a novel response to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Biochem. Biophys. Res. Commun. 289, 499-506.
65. Mansuri, M.S., Singh, M., Jadeja, S.D., Gani, A.R., Patel, R., Dwivedi, M., *et al*., 2014. Could ER stress be a major link between oxidative stress and autoimmunity in Vitiligo? Pigmentary Disorders. 1, 123.
66. Mao, Z., Hine, C., Tian, X., Van Meter, M., Au, M., Vaidya, A., *et al*., 2011. SIRT6 promotes DNA repair under stress by activating PARP-1. Science. 332, 1443-1446.
67. Masutani, M., Nakagama, H., Sugimura, T., 2003. Poly(ADP-ribose) and carcinogenesis. Genes Chromosomes Cancer. 38, 339-348.
68. Midorikawa, R., Takei, Y., Hirokawa, N., 2006. KIF4 motor regulates activity-dependent neuronal survival by suppressing PARP-1 enzymatic activity. Cell. 125, 371-383.
69. Morales, J., Li, L., Fattah, F.J., Dong, Y., Bey, E.A., Patel, M., *et al*., 2014. Review of poly(ADP-Ribose) polymerase (PARP) mechanisms of action and rationale for targeting in cancer and other diseases. Crit. Rev. Eukaryot. Gene Expr. 24, 15-28.
70. Mortusewicz, O., Amé, J.C., Schreiber, V., Leonhardt, H., 2007. Feedback-regulated poly(ADP-ribosyl)ation by PARP-1 is required for rapid response to DNA damage in living cells. Nucleic Acids Res. 35, 7665-7675.

71. Muñoz-Gámez, J. A., Rodríguez-Vargas, J. M., Quiles-Pérez, R., Aguilar-Quesada, R., Martín-Oliva, D., de Murcia, G., *et al*., 2009. PARP-1 is involved in autophagy induced by DNA damage. Autophagy. 5, 61-74.
72. Näär, A. M., Lemon, B. D., Tjian, R., 2001. Transcriptional coactivator complexes. Annual review of biochemistry. 70, 475-501.
73. Nalabothula, N., Al-jumaily, T., Eteleeb, A.M., Flight, R.M., Xiaorong, S., Moseley, H., *et al.,* 2015. Genome-wide profiling of PARP1 reveals an interplay with gene regulatory regions and DNA methylation. PLoS One. 10, e0135410.
74. O'Donnell, A., Yang, S.H., Sharrocks, A.D., 2013. PARP1 orchestrates variant histone exchange in signal-mediated transcriptional activation. EMBO J. 14, 1084-1091.
75. Oei, S.L., Shi, Y., 2001. Poly (ADP-ribosyl)ation of transcription factor Y in Yang 1 under conditions of DNA damage. Biochem. Biophys. Res. Commun. 285, 27-31.
76. Oka, J., Ueda, K., Hayaishi, O., Komura, H., Nakanishi, K., 1984. ADP-ribosyl protein lyase. Purification, properties, and identification of the product. Journal of Bio Chemistry. 259, 986– 995.
77. Oka, S., Kato, J., Moss, J., 2006. Identification and Characterization of a Mammalian 39-kDa Poly(ADP-ribose) Glycohydrolase. Journal of Biological Chemistry. 281, 705-713.
78. Okada, H., Inoue, T., Kikuta, T., Kato, N., Kanno, Y., Hirosawa, N., *et al*., 2008. Poly(ADP-ribose) polymerase-1 enhances transcription of the profibrotic CCN2 gene. J. Am Soc. Nephrol. 19, 933-942.
79. Ong, C.T., Van Bortle, K., Ramos, E., Corces, V.G., 2013. Poly(ADP-ribosyl)ation regulates insulator function and intrachromosomal interactions in *Drosophila*. Cell. 155, 148-159.
80. Ouararhni, K., Hadj-Slimane, R., Ait-Si-Ali, S., Robin, P., Mietton, F., Harel-Bellan, A., 2006. The histone variant mH2A1.1 interferes with transcription by down-regulating PARP-1 enzymatic activity. Genes Dev. 20, 3324-3336.

81. Patel, A.G., Sarkaria, J.N., Kaufmann S.H., 2011. Nonhomologous end joining drives poly(ADP-ribose) polymerase (PARP) inhibitor lethality in homologous recombination-deficient cells. Proc. Natl. Acad. Sci. USA. 108, 3406-3411.
82. Pinnola, A., Naumova, N., Shah, M., Tulin, A.V., 2007. Nucleosomal Core Histones Mediate Dynamic regulation of Poly(ADP-ribose) Polymerase 1 Protein Binding to Chromatin and Induction of Its enzymatic Activity. J. Biol. Chem. 282, 32511-32519.
83. Poirier, G. G., De Murcia, G., Jongstra-Bilen, J., Niedergang, C., Mandel, P., 1982. Poly (ADP-ribosyl) ation of polynucleosomes causes relaxation of chromatin structure. Proceedings of the National Academy of Sciences. 79,3423-3427.
84. Reinberg, A.R., 1998. Chalcogenide memory cell with a plurality of chalcogenide electrodes. *U.S. Patent No. 5,789,758*. Washington, DC: U.S. Patent and Trademark Office.
85. Robu, M., Shah, R.G., Petitclerc, N., Brind'Amour, J., Kandan-Kulangara, F., Shah, G.M., 2013. Role of poly(ADP-ribose) polymerase-1 in the removal of UV-induced DNA lesions by nucleotide excision repair. Proc. Natl. Acad. Sci. USA. 110, 1658-1663.
86. Rosenthal F., Feijs KLH., Frugier E., Bonalli M., Forst AH., Imhof R., *et al.*, 2013. Macrodomain-containing proteins are new mono-ADP-ribosylhydrolases. Nat. Struct. Mol. 20, 502-509.
87. Ruf, A., Rolli, V., de Murcia, G., Schulz, G. E., 1998. The mechanism of the elongation and branching reaction of poly (ADP-ribose) polymerase as derived from crystal structures and mutagenesis. Journal of Molecular Biology. 278, 57-95.
88. Rulten, S.L., Cortes-Ledesma, F., Guo, L., Iles, N.J., Caldecott, K.W., 2008. APLF (C2orf13) is a novel component of poly (ADP-ribose) signaling in mammalian cells. Mol. Cell. Biol. 28, 4620-4628.
89. Schreiber, V., Dantzer, F., Ame, J.C., de Murcia, G., 2006. Poly(ADP-ribose): novel functions for an old molecule. Nat. Rev. Mol. Cell Biol. 7, 517-528.

90. Schreiber, V., Molinete, M., Boeuf, H., de Murcia, G., Menissier-de Murcia, J., 1992. The human poly (ADP-ribose) polymerase nuclear localization signal is a bipartite element functionally separate from DNA binding and catalytic activity. EMBO J. 11, 3263.
91. Seimiya, H., 2006. The telomeric PARP, tankyrases, as targets for cancer therapy. Br. J. Cancer 94, 341-345.
92. Smith, S., Giriat, I., Schmitt, A., de Lange, T., 1998. Tankyrase, a poly(ADP-ribose) polymerase at human telomeres. Science. 282, 1484-1487.
93. Son, Y.O., Wang, X., Hitron, J.A., Zhang, Z., Cheng, S., Budhraja, A., *et al*., 2011. Cadmium induces autophagy through ROS-dependent activation of the LKB1–AMPK signaling in skin epidermal cells. Toxicol. Appl. Pharmacol. 255, 287-296.
94. Sperandio, S., Poksay, K., De Belle, I., Lafuente, M. J., Liu, B., Nasir, J., Bredesen, D. E., 2004. Paraptosis: mediation by MAP kinases and inhibition by AIP-1/Alix. Cell Death & Differentiation. 11, 1066-1075.
95. Timinszky, G., Till, S., Hassa, P.O., Hothorn, M., Kustatscher, G., Nijmeijer, B., *et al*., 2009. A macrodomain-containing histone rearranges chromatin upon sensing PARP1 activation. Nat. Struct. Mol. Biol. 16, 923-929.
96. Tulin, A., Spradling, A., 2003. Chromatin loosening by poly(ADP-ribose) polymerase (PARP) at Drosophila Puff Loci. Science. 299, 560-562.
97. Tutt, A., Ashworth, A., 2002. The relationship between the roles of BRCA genes in DNA repair and cancer predisposition. Trends Mol. Med. 8, 571-576.
98. Virág, L., Szabó, C., 2002. The therapeutic potential of poly (ADP-ribose) polymerase inhibitors. Pharmacological reviews. 54, 375-429.
99. Vyas, S., Chesarone-Cataldo, M., Todorova, T., Huang, Y.H., Chang, P., 2013. A systematic analysis of the PARP protein family identifies new functions critical for cell physiology. Nat. Commun. 4, 2240.
100. Wang, Y., Li, X., Wang, L., Ding, P., Zhang, Y., Han, W., *et al*., 2004. An alternative form of paraptosis-like cell death, triggered by TAJ/TROY and enhanced by PDCD5 overexpression. Journal of cell science. 117, 1525-1532.

101. Weaver, A. N., Yang, E. S., 2013. Beyond DNA repair: additional functions of PARP-1 in cancer. Trials. 9, 10.

102. Wu, T., Wang, X.J., Tian, W., Jaramillo, M.C., Lau, A., Zhang, D.D., 2014. Poly (ADP-ribose) polymerase-1 modulates Nrf2-dependent transcription. Free Radic. Biol. Med. 67, 69-80.

103. Xie, S., Mortusewicz, O., Ma, H.T., Herr, P., Poon, R.R., Helleday, T., *et al.*, 2015. Timeless interacts with PARP-1 to promote homologous recombination repair. Mol. Cell. 60, 163-167.

104. Ye'lamos, J., Farres, J., Llacuna, L., Ampurdanes, C., Martin-Caballero, J., 2011. PARP-1 and PARP-2: new players in tumour development. Am. J. Cancer Res. 1, 328-346.

105. Yu, S. W., Wang, H., Poitras, M. F., Coombs, C., Bowers, W. J., Federoff, H. J., 2002. Mediation of poly(ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. Science. 297**,** 259–263.

106. Zhang, T., Berrocal, J.G., Yao, J., DuMond, M.E., Krishnakumar, R., Ruhl, D.D., 2012. Regulation of poly(ADP-ribose) polymerase-1-dependent gene expression through promoter-directed recruitment of a nuclear NAD^+ synthase. J. Biol. Chem. 287, 12405-12416.

107. Zhao, H., Sifakis, E.G., Sumida, N., Millán-Ariño, L., Scholz, B.A., Svensson, J.P., *et al.*, 2015. PARP1-and CTCF-mediated interactions between active and repressed chromatin at the lamina promote oscillating transcription. Mol. Cell. 59, 984-997.

Objectives

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.