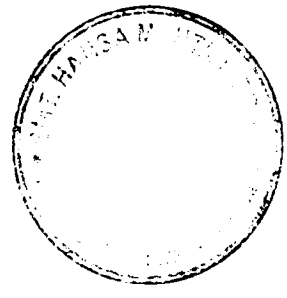

Synopsis

Introduction

In multicellular organisms, cell proliferation and cell death are carefully coordinated to control the number and organization of cells in tissues and organs. An imbalance in these processes can result in several diseases. One such disease is cancer that arises from the accumulation of cells due to hyper proliferation or reduced cell death. Successful prevention and treatment of various such diseases entails a fine understanding of normal regulation of cell death and cell survival. In addition to the two distinct and well characterized forms of cell death i.e., necrosis and apoptosis, there are programs that use death machinery in a specially defined and carefully choreographed manner. Paraptotic cell death is one such important physiological mode of cell death (Lockshin and Zakeri, 2004). It can occur concomitantly with apoptosis or can function as an alternative process when apoptosis is impaired.

Apoptosis is characterized by certain biochemical and morphological changes which include early translocation of phosphatidyl serine (PS) to the outer leaflet of plasma membrane, caspase activation, mitochondrial membrane potential (MMP) changes, oligonucleosomal DNA fragmentation and apoptotic body formation (Hengartner, 2000). Paraptosis, on the contrary, is characterized by cytoplasmic vacuolization, mitochondrial swelling, large scale DNA fragmentation and absence of caspase activation (Sperandio *et al.*, 2000; Katoch *et al.*, 2002). Mitochondria and nucleus have crucial roles in this cell death pathway. Release of apoptosis-inducing factor (AIF) from mitochondria signaled by nuclear poly (ADP-ribose) polymerase (PARP) appears to be an important event during paraptotic cell death. AIF is also indispensable for the cell death inducers which evade PARP. Nevertheless AIF is demonstrated to have an important role in mitochondrial functioning. Thus AIF acts as a double edged sword, since its translocation from mitochondria to nucleus not only causes DNA fragmentation in the nucleus but also impairs the mitochondrial electron transport chain. Hence, understanding this crucial signaling between mitochondria and nucleus during cell death becomes important. This information can provide better therapeutic targets for several disease conditions that are associated with cell death induced by nuclear DNA damage and mitochondrial dysfunction.

D. discoideum often referred to as "slime mold", is the model organism used in this study. It is one of the simplest studied eukaryotes that possesses multicellularity. Vegetative stage and developmental forms in *D. discoideum* are temporally separated. The relatively simple pattern of development in this organism facilitates study of

developmental cell death. It has been observed by many researchers that *D. discoideum* demonstrates cell death mechanisms similar to that seen in some of the higher eukaryotes. Also, absence of caspases (Olie *et al.*, 1998) makes this organism a suitable model system to study paraptotic programmed cell death. Nine potential PARP genes have been identified in *D. discoideum* (Otto *et al.*, 2005). However, information on the role of PARP in *D. discoideum* cell death is limited. The present study is an attempt to explore the role of PARP and its downstream effectors during *D. discoideum* cell death induced by different stress conditions such as UV-C, staurosporine and starvation. Other part of this study deals with down regulation of AIF, an important downstream mediator of PARP induced cell death in the absence of caspases and deciphering its role in cell death.

Objectives

- Role of PARP in UV-C induced cell death, and developmental changes in *D. discoideum*
- Role of PARP in staurosporine induced cell death, and developmental changes in *D. discoideum*
- Role of PARP in starvation induced cell death and development in *D. discoideum*
- Role of AIF in *D. discoideum* cell death and development

I. Role of PARP in UV-C induced cell death, and developmental changes in *D. discoideum*

UV-C induced cell death in *D. discoideum* is PARP mediated

Radiation and chemical mutagens are direct DNA damaging agents. Frequently used radiation in biological studies is Ultra Violet (UV) radiation and UV-C is efficiently attenuated by the earth's atmosphere. However, exposure of mammalian cells to UV-C radiation results in DNA damage and generation of reactive oxygen species (ROS) (Cadet *et al.*, 1997), which in turn perturb the redox balance of the cell and result in oxidative stress. While the oxidative stress induced by ionizing radiation and

alkylating agents accounts for the DNA damage incurred by these agents, ROS are not the major cause of DNA damage in UV-C irradiated cells. UV-C also leads to distortion in DNA double helical structure which is sufficient to activate PARP (Rouleau *et al.*, 2004).

UV-C induces dose dependent cell death in *D. discoideum*

Cell death was induced by subjecting *D. discoideum* cells to different doses of UV-C irradiation i.e., 0-130 J/m^2 and cell death was monitored by trypan blue exclusion method at different post irradiation periods (1-22 hours). Early cell death was observed with 65 J/m^2 and 130 J/m^2 of UV-C irradiation. In case of 26 J/m^2 and 39 J/m^2 of UV-C treated cells, early cell death was less and the percent cell death increased after 7 hours. These results suggest that 65 J/m^2 and 130 J/m^2 of UV-C stress could be necrotic, whereas 26 J/m^2 and 39 J/m^2 of UV-C stress could be apoptotic for *D. discoideum* cells.

PS-PI dual staining distinguishes between apoptotic & necrotic cell death. These results confirmed 39 J/m^2 of UV-C stress to be apoptotic dose and 130 J/m^2 of UV-C stress to be necrotic dose to *D. discoideum* cells.

PARP activation and its inhibition by benzamide under UV-C stress

PARP activity was assayed at various time points using indirect immunoflourescence technique. 39 J/m^2 UV-C treated cells showed the highest PARP activity at 5 minutes which then declines, and reaches to basal level at 15 minutes. 130 J/m^2 of UV-C treated cells showed peak of PARP activity at 2 minutes and then it declined and reached to normal levels after 10 minutes. Benzamide pre-treated cells showed inhibition of the PARP activity.

NAD$^+$ and ATP levels reduce during PARP mediated cell death

PARP utilizes NAD$^+$ as a substrate and acute reduction in the NAD$^+$ is followed by a drop in cellular ATP levels. The depleted NAD$^+$ and ATP could be one of the several factors responsible for downstream events. Two-fold decrease in NAD$^+$ levels, as estimated by enzyme cycling method, was observed after 1 hour in 39 J/m^2 UV-C stressed cells and similar trend was seen with ATP level as measured using HPLC. This is a supportive evidence for PARP activation downstream to DNA damage

induced by UV-C. Benzamide pretreatment prevents this reduction by inhibiting PARP activity.

PARP mediated cell death involves mitochondrial changes

39 J/m^2 of UV-C treated cells showed significant decrease in MMP (Mitochondrial membrane potential) after 4 hours, whereas 130 J/m^2 of UV-C treated cells showed significant increase in MMP after 2 hours. Benzamide rescued the cells from these MMP changes by inhibiting PARP. MMP changes were monitored using DiOC6.

AIF translocation and DNA fragmentation

Oligonucleosomal DNA ladder formation is one of the hallmarks of apoptosis, however UV-C treated cells did not show oligonucleosomal DNA fragmentation signifying that not caspases but other factors are employed by these cells to execute cell death. These UV-C stressed cells however were TUNEL positive implying that they exhibit large scale DNA fragmentation, a characteristic feature of caspase independent cell death.

With 130 J/m^2 UV-C treated cells, AIF release was observed after 2 hours using immunofluorescence technique. However, benzamide pretreatment did not show inhibition of AIF nuclear translocation. In 39 J/m^2 of UV-C treated cells AIF was released from mitochondria after 4 hours and it also showed nuclear localization. Inhibition of PARP by benzamide pretreatment also inhibited AIF release. Thus, our results suggest that AIF release is downstream to PARP activation and its translocation to nucleus causes large scale DNA fragmentation.

Role of caspases during PARP initiated cell death

Caspase is the most extensively studied downstream executioner of programmed cell death. Programmed cell death and caspases have been considered to go hand in hand until recently. However, PARP mediated cell death is a mushrooming aspect of cell death studies which is devoid of the contribution of caspases (Hong *et al.*, 2004). Predictably pretreatment with broad caspase inhibitors did not affect the PI staining in UV-C treated *D. discoideum* cells.

Role of MEK during PARP initiated cell death

The role of MEK in UV-C induced tumor progression has been addressed (Shaw *et al.*, 2004). Involvement of MEK during oxidative stress induced cell death in various mammalian cell lines namely human neuroblastoma SH-SY5Y cells (Saeki *et al.*, 2000) is well known. However, involvement of MEK in UV-C induced paraptosis has not been addressed. Results presented in this study show that MEK does not have a role in PARP mediated paraptotic cell death induced by UV-C as pretreatment with MEK inhibitor did not affect the PI staining in UV-C treated *D. discoideum* cells.

Effect of UV-C on *D. discoideum* growth and the role of PARP

There was a dose dependent increase in the lag phase with UV-C dose (10.4-130 J/m^2) i.e., with increase in UV-C dose there was an increase in the lag phase and consequently a late entry into the log phase. Cells exposed to higher doses of UV-C (130 J/m^2) show 50% reduction in viability and have longer lag phase (~100 hours). Pretreatment of cells with benzamide showed significant rescue in UV-C induced changes in the growth. These results suggest that PARP activation after UV-C stress affects the growth of *D. discoideum* cells.

UV-C induced changes in *D. discoideum* development and the role of PARP

Signal transduction is involved in nearly all physiological events, and defects in signal transduction pathways often give rise to disease conditions. These processes are difficult to study in complex multicellular organisms. There are reports which show that certain signaling strategies have been conserved throughout eukaryotic evolution. *D. discoideum* being a eukaryote that stands at the transition point of unicellularity and multicellularity is an excellent model system to study various signal transduction pathways (Mir *et al.*, 2007) that can later be extrapolated to mammalian systems. Also, counter parts of mammalian G protein-linked signal transduction events essential for chemotaxis, cell aggregation, morphogenesis, gene expression, and pattern formation are found in *Dictyostelium*. Chemotaxis process in *Dictyostelium* resembles chemokine mediated chemotactic movement of leukocytes towards infection (Van and Devreotes, 1999).

Effect of UV-C on development

The effect of UV-C on development also reflected dose dependency as seen in cell death experiment however, the effect was more dramatic. 10.4 J/m^2 UV-C irradiated cells could develop, however, cells exposed to 13 J/m^2, 65 J/m^2 and 130 J/m^2 UV-C failed to enter the development. Also 10.4 J/m^2 UV-C treated cells displayed delayed development compared to control cells.

UV-C induced PARP activation

PARP was assayed at various time points (2 and 5 minutes) post UV-C stress and 10.4 J/m^2 and 13 J/m^2 UV-C treated cells showed significant increase in PARP activity at 2 minutes which then declined after 5 minutes. Benzamide pretreated cells did not show activation of PARP after 2 minutes UV-C treatment.

PARP inhibition affects UV-C induced changes in development

Pretreatment of cells with benzamide showed rescue in UV-C induced changes in the developmental delay observed with 10.4 J/m^2 UV-C irradiation. These results suggest that PARP activation after UV-C stress affects the development of *D. discoideum* cells.

UV-C irradiated cells form defective spores

Cells irradiated with 10.4 J/m^2 of UV-C undergo delayed development followed by delayed germination compared to control spores. This could be due to lesser number of cells combining to form a smaller fruit as the number of fruiting bodies is regained on increasing the cell density. 10.4 J/m^2 of UV-C irradiated cells with benzamide pretreatment did not show any significant change compared to 10.4 J/m^2 of UV-C treated cells. This result is intriguing as PARP inhibition corrects the growth defects induced by UV-C however, when the same cells' spores are put for germination, PARP inhibition did not show any change. This implicates that PARP may not be involved in increasing the dormancy of spores induced by UV-C. Interestingly, cells germinated from spores did not show any significant DNA damage signifying that the basal PARP activity may be sufficient to repair the DNA damage induced by UV-C.

This response to PARP inhibition during UV-C induced changes in *D. discoideum* growth and development differs from our results on oxidative stress response (Rajawat *et al.*, 2007). The spores formed under oxidative stress exhibited delayed

germination compared to benzamide pretreated stressed cells suggesting that PARP inhibition during oxidative stress not only corrects the delayed development but also retains the normal germination time of spores (Rajawat *et al.*, 2007). Thus *D. discoideum* cells respond to oxidative and UV-C stress in different manner.

Effect of UV-C on signaling during *D. discoideum* development

Despite of high resistance, *D. discoideum* cells exhibited complete block in the development when exposed to very mild dose of 13 J/m^2 UV-C. However, mixing UV-C exposed cells with healthy cells evade the developmental block induced by irradiation. Recovery of development of UV-C irradiated cells in the mixing experiment notifies that UV-C induced developmental defect is probably due to impaired extracellular signaling where either the production or the response to the signal is being affected. cAMP being the key component of chemotaxis, it controls developmentally regulated gene expression and hence development. Any defect in generation of cAMP pulses or its sensing would affect aggregation and thus leads to delay or arrest in development. This assumption is supported by complete restoration of development of UV-C exposed cells by exogenous cAMP. Also, cAMP levels in UV-C treated cells are found to be decreased in a dose dependent manner. These experiments confirm that UV-C affects development by impairing cAMP mediated signaling.

cAMP levels regulate expression of aggregative genes and adenylyl cyclase A (ACA), as well as its activation and adaptation. These long term responses of cAMP are mediated via cAR1 receptor. Gene expression studies show that UV-C alters the expression of *cAR1* and also that of *ACA*. Chemotaxis experiment done with exogenous cAMP connotes that UV-C treated cell failed to chemotax towards cAMP. Wang and Kuspa (1997) showed that cells with disrupted *acaA* do not secrete cAMP and are unable to chemotactically signal each other unless pulsed with extra cellular cAMP or genetically engineered to over express the catalytic subunit of the cAMP-dependent protein kinase, PKA. Thus chemotaxis experiment substantiates our gene expression studies and implies that initial sensing and the subsequent production of cAMP are getting affected under UV-C irradiation. Independent or cumulative effect of these two factors may seize the process of development.

In addition to the temporal effect on development, the fruiting bodies formed after UV-C treatment are smaller and lesser in number. This observation prompted us to

study the expression of counting factor (CF) complex. Alteration in the expression of countin 2 protein, a component of the ~450 kDa counting factor complex, validated our presumption that UV-C irradiated cells also have a compromised cell counting mechanism along with altered extracellular signaling. Besides this CF has a noteworthy role in regulating signal transduction pathways leading to cAMP pulses and pseudopod formation. Hence changes in CF expression may affect cAMP production and consequently overall development. However, the reverse may not be true.

It has also been reported that NO generating compounds inhibit cAMP pulses during *D. discoideum* aggregation stage (Tao *et al.*, 1996; Katoch and Begum, 2003). UV-A and UV-B irradiation induces NO generation in keratinocytes which further stimulates the melanogenesis (Romero-Graillet *et al.*, 1997). Additionally, increased NO inhibits some isoforms of adenylate cyclases (especially AC5 and AC6) in mammalian cells (Freeman *et al.*, 2004). Interestingly probing the development of UV-C treated cells with iNOS inhibitor, LNio6 and estimation of NO during development supports this notion of UV-C inducing NO generation *via* iNOS.

Thus, UV-C irradiation affects cAMP pulses and expression of *countin2, aca and cAR1* via increased production of NO. These studies emphasize that despite of high resistance power against oxidative stress, gamma and UV radiation, mild dose of UV-C was sufficient to destabilize *D. discoideum* to form multicellular structure by interfering the cAMP signaling machinery.

II. Role of PARP in staurosporine induced cell death, and developmental changes in *D. discoideum*

Role of PARP in staurosporine induced cell death in *D. discoideum*

Staurosporine (STS) is a bacterial alkaloid obtained from *Streptomyces staurosporeus,* with inhibitory activity against fungi and yeast. It is a cell permeable inhibitor of protein kinases including protein kinase C.

STS is known to induce cell death in many mammalian cell types by both caspase dependent and independent apoptotic pathways. STS mediated caspase independent cell death mimics mitochondrial death pathway in which Bax and Bak proapoptotic proteins play key roles (Omura *et al.*, 1977). It causes limited lysosomal destabilization which releases several proteases like cathepsin B, D, and L; amongst

which cathepsin D (Cat D), an aspartate protease, is able to act at neutral pH owing to its stabilized active conformation achieved by binding with target cytosolic proteins. Release of Cat D activates Bax by proteolytic cleavage leading to limited permeabilization of the outer mitochondrial membrane, which in turn results in the release of AIF from the inter membrane space of mitochondria to the cytosol. Once released in to the cytosol, AIF translocates to the nucleus where it brings about large scale DNA fragmentation and ultimately paraptotic cell death (Bidere *et al.*, 2003).

Induction of paraptosis by staurosporine

D. discoideum cells when treated with staurosporine (100-500 nM) and it is observed that as the concentration of STS increases from 100 to 500 nM % live cells decrease. This trend continues till 8 hours with significant decrease in cell number at 2 hours with 500 nM STS. Thus, for further experiments a lower dose of 400 nM was selected. Cells treated with 400 nM STS showed PS exposure at 4 hours whereas the plasma membrane integrity was lost at 6 hours as shown by PI staining, while 500 nM STS treated cells were both PS-PI positive at 4 hours. These cells showed significant reduction in MMP at 3 hours and AIF translocation to nucleus was observed at 4 hours.

Staurosporine induced paraptosis in *D. discoideum* cells involves cathepsin D

When the cells were pre-incubated with pepstatin A, a cathepsin D inhibitor, significant rescue was seen in all the above mentioned parameters, confirming the role of cathepsin D in the release of AIF from mitochondria and subsequent cell death induced by 400 nM STS. STS induced cell death involves Bcl-2 family proteins (Omura *et al.*, 1977). However, it is recently reported that *Dictyostelium* cells do not express Bcl-2 family proteins which suggests the possibility of an alternative pathway being operated during staurosporine stress to induce mitochondrial changes through cathepsin D (Lam *et al.*, 2007).

Staurosporine induced cell death is independent of PARP activity

Though the molecular mechanism of staurosporine induced caspase independent cell death is well established (Daugas *et al.*, 2000), the role of PARP in the cascade of events led by staurosporine is not yet known. Our PARP activation studies suggest that PARP was not activated by 400 nM staurosporine at 5-20 minutes interval. Also,

PARP inhibition by benzamide did not show rescue in the MMP changes induced by staurosporine. These results point out that PARP might not be involved in staurosporine induced cell death.

***D. discoideum* development is not dependent on cathepsin D activity**

Effect of STS on the development of *D. discoideum* cells in the presence and absence of PARP and cathepsin D was also studied. It was observed that the number of developing bodies was less in STS treated cells however, the development occurred within the normal time period i.e., fruiting bodies were seen at 24 hours. Cathepsin D inhibition could restore the number of developing bodies to normal in STS treated *D. discoideum* cells. This could be due to the reduction in the number of dying cells thereby an increase in the effective number of cells taking part in the developmental process.

III. Role of PARP in starvation induced cell death and development in *D. discoideum*

Starvation induces a time dependent increase in cell death

Studies with conditioned medium have shown that *Dictyostelium* cells are capable of showing paraptotic cell death even in vegetative form (Arnoult *et al.*, 2001), which is also supported by our oxidative stress (Rajawat, 2010) and staurosporine studies. *D. discoideum* cells, when subjected to starvation in SB (Sorenson's Buffer) buffer showed time dependent increase in cell death. There was significant decrease in number of live cells compared to the number of cells in HL5 medium. Significant number of dead cells could not be observed during initial 1-4 hours possibly due to the cells taking up necrotic type of cell death in the initial 1-4 hours, while more number of dead cells could be seen at 5-7 hours of starvation.

***D. discoideum* cells show two modes of cell death under starvation**

The mode of cell death was determined using PS-PI dual staining kit. It was observed that under starvation *D. discoideum* cells underwent cell death in two phases. In the early phase, starved cells showed necrotic cell death as PS exposure as well as PI staining was observed simultaneously at 2 hours whereas, the later phase of cell death was paraptotic as PS exposure was observed at 5 hours, but it was not accompanied by PI staining indicating that the plasma membrane integrity was not lost.

Reduced ATP levels induced by starvation could lead to a decline in function of the ATP-dependent ion (Na^{2+} and Ca^{2+}) pumps on the plasma membrane. Perturbation of intracellular ion homeostasis can result in mitochondrial dysfunction and diminished ATP production (Zong & Thompson, 2006). Hence MMP was monitored and it was observed that starved cells showed changes in MMP at 2, 4 and 6 hours and resulting in significant cell death in a time dependent manner.

Interestingly, at 6 hours of starvation there was an increase in the MMP of starved cells compared to control cells. This increase could be due to shift of cellular respiration from state 3 to state 4 as observed in case of reduced cellular ATP (Cipriani *et al.*, 2005) in HeLa cells. These changes in MMP at different time points under starvation may be accounted for two types of cell death processes namely necrosis and paraptosis taken up by the cells.

Protease involvement in starvation induced cell death

To elucidate the molecular mechanism of starvation induced cell death, protease inhibition studies were done. Protease involvement was observed particularly in the initial 2-7 hours of starvation.

The cells when pre-incubated with the protease inhibitors showed rescue in MMP changes at two time points and also in the cell death. These results implicate that proteases function upstream to MMP changes during starvation induced cell death. Cathepsin D is a lysosomal protease that is known to be active at the cytosolic pH and could serve as the protease involved in starvation induced cell death. Hence experiments were done using cathepsin D inhibitor pepstatin A. Cathepsin D inhibition showed a partial rescue in cell death. This was also reflected in its effect on MMP changes which were prevented significantly at 2 and 4 hours after starvation induction. Pepstatin A pretreated cells when starved, showed PS exposure while cells subjected to starvation without pepstatin A pretreatment showed both PS exposure as well as PI staining. This suggests that cathepsin D, one of the proteases involved in starvation induced cell death, when inhibited shifts the necrotic mode of cell death to paraptotic mode. It has been reported that ATP depletion affects the ATP dependent Ca^{2+} pumps thereby leading to a rise in the cytosolic Ca^{2+} levels. Calpains activated due to Ca^{2+} overload contribute to the lysosomal membrane permeability (LMP), which can lead to release of lysosomal enzymes including cathepsins and ultimately necrotic cell death (Yamashima, 2004; Lum *et al.*, 2005). Studies with calpain

inhibitor also showed protection in starvation induced cell death. Hence, cell dismantling during starvation induced cell death could be attributed to calpains and/or lysosomal proteases, as caspases are absent in *D. discoideum* (Bouffay *et al.*, 2004). Interestingly pepstatin A preincubation did not affect *D. discoideum* development which indicates that cathepsin D may not have significant role to play in developmental cell death.

Second phase of starvation induced cell death requires PARP

To explore the role of PARP in starvation induced cell death the cells were pre incubated with benzamide followed by trypan blue test at different time points till 24 hours. PARP inhibition had no significant effect during the first 4 hours of starvation. However, inhibition of PARP was found to be protective for cells at 5-6 hours after starvation induction. ROS are known to be generated during nutrient depletion (Zong & Thompson, 2006). These ROS can lead to DNA damage that could serve as a signal for PARP activation. Starving *D. discoideum* cells also showed ROS dependent DNA damage as assayed by immunofluorescence technique targeting H2AX. Effect of PARP inhibition on starvation induced cell death is also reflected in PS exposure and PI staining.

PARP inhibition prevents changes in MMP at 4 hours suggesting that though PARP may not be the sole mediator, it is important for the execution of starvation induced cell death.

Levels of NAD^+ were maintained even after 1 hour of starvation induction. This further supports that PARP activation does not occur immediately after starvation induction. This was confirmed by benzamide experiments. However, benzamide pretreated cells showed significant rescue in NAD^+ levels that were reduced at 5 hours after starvation induction.

***D. discoideum* development and PARP**

The role of PARP in *D. discoideum* development was investigated by its inhibition with benzamide. Benzamide (1.0, 2.0 and 3.0 mM) did not show any effect on development. However, benzamide at 4 mM dose delayed the transition from tight aggregate (TA) to slug by 3-4 hours. This suggests the likely role of PARP during development. Interestingly, 3.0 and 4.0 mM benzamide treated *D. discoideum* cells showed abnormal fruiting body with bigger size fruits.

IV. Role of AIF in *D. discoideum* cell death and development

Down-regulation of AIF by antisense in *D. discoideum*

Susin *et al.,* 1999 reported that AIF, a mitochondrial inter-membrane space flavoprotein, can induce isolated nuclei to acquire features of paraptosis such as peripheral chromatin condensation and large scale DNA fragmentation. The cell death pathway initiated by PARP activation is mediated by AIF in paraptosis. Activated PARP can induce the translocation of AIF from mitochondria to the nucleus, and further lead to downstream events such as nuclear condensation, PS exposure and cell death. Thus AIF is an essential downstream effector of the paraptotic cell death events (Yu *et al.,* 2002). Nevertheless AIF also has a role in mitochondrial functions. Cerebellar granule cells from Harlequin (Hq) mice in which AIF expression is reduced markedly are susceptible to oxidative stress and exhibit short life span. Due to the vital role of AIF in a normal cell, AIF knock outs are not viable (Arnoult *et al.,* 2001) and hence it would be interesting to investigate the role of AIF in paraptotic cell death by its downregulaion. Down regulation of AIF by antisense has been attempted in this study. The antisense clones under inducible and constitutive promoters (ecmB and pTX respectively) have been constructed and transformed into *D. discoideum* cells. Resultant reduction in AIF and the subsequent physiological effects would delineate the role of AIF in *D. discoideum* cell death and development; as well as its vital role in mitochondrial function. Our preliminary results with the constitutively downregulated AIF cells suggest that AIF is important for *D. discoideum* development.

In conclusion, present results provide insights for designing novel drugs to control diseases involving PARP and AIF, which are also involved in important cellular processes other than cell death. As Calpains and cathepsin D are involved in cell dismantling during paraptotic cell death they serve as surrogate caspases. This study would help to identify better targets for inhibiting or inducing paraptotic cell death during diseased conditions. Also this study highlights the fact that *D. discoideum,* being a lower eukaryote, has different mechanisms to respond to different stresses similar to mammalian systems emphasizing the importance of this organism as a model for various studies.

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