Concluding Remarks

Caspases may be indispensable for typical apoptotic morphology but they are not the sole determinants of life and death decisions in programmed cell death (PCD). Evidences are accumulating that PCD can occur in complete absence of caspases. One such form of caspase independent cell death is termed as paraptosis. Paraptosis also appears to occur during development of the nervous system, as well as in some cases of neurodegenerative disorders (Stoka *et al.*, 2007). Paraptosis has been described to be mediated by several proteins or factors; Poly(ADP-ribose) polymerase (PARP) being one of them.

It appears that the mitochondria and nucleus have crucial roles in cell death. Understanding the signalling between these two organelles during cell death might lead to more options to develop new therapeutic targets for several diseases that are associated with cell death induced by mitochondrial dysfunction and nuclear DNA damage. The molecular mechanisms responsible for PARP-1-dependent cell death involve the release of AIF from mitochondria and translocation to the nucleus. Poly(ADP-ribosyl)ation of cellular proteins might activate the relocation of AIF. However, the factors and the mechanisms of mitochondrial AIF expulsion remain to be elucidated. The identification of the AIF-binding factors that trigger relocation from the mitochondria to the nucleus and contribute to chromatin condensation in the nucleus might be particularly important in the development of new pharmaceutical targets. PARP-1-dependent cell death was noted first in the CNS, but was followed rapidly by the appreciation that this program also operated in many other organ systems.

Several studies suggest that mitochondria play a central role in the execution of programmed cell death. Oxidative stress has been reported to cause mitochondrial dysfunction directly by promoting rapid loss of MMP (mitochondrial membrane potential). When cells take up paraptosis, mitochondria undergo an initial priming phase associated with hyperpolarization which further leads to an effector phase, during which mitochondria swell and release proapoptotic proteins (Cipriani *et al.*, 2005). NAD^+ being a substrate for PARP, its supplementation showed increased

PARP activation in a dose dependent manner, whereas pretreatment with benzamide (PARP inhibitor) and gallotannin (PARG inhibitor) showed decreased PARP activity and followed by rescue in cell death induced by cadmium and cumene H_2O_2. Although all these agents i.e. benzamide, gallaotannin and NAD^+ had opposing effect on PARylation. This implies that PARP activation may not be the actual culprit leading to cell death. Significant rescue in cell death with exogenous NAD^+ despite of increasing PARP activity further project PARP as an **"Innocent killer protein"** (as its actual role is to recruit DNA repair machinery upon DNA damage). Interestingly inhibition of calpain (ALLN), did not affect the oxidative stress induced PARP activation, suggesting calpain activation is a downstream event in cell death cascade. Furthermore, NAD^+, benzamide, and gallotannin pretreated *D. discoideum* cells could prevent NAD^+ depletion followed by MMP changes induced by oxidative stress. In other words, NAD^+ depletion is a turning point during entire cell death cascade, suggesting NAD^+ to be the **"Currency coin"** during nuclear-mitochondrial cross talk; thus preventing NAD^+ depletion could block the downstream events leading to cell death. Thus this study sheds light on the current use of NAD^+, benzamide, gallotannin and ALLN as pharmacological agents for treatment of diseases associated with oxidative stress induced PARP mediated cell death.

D. discoideum lacks caspases while it interestingly possesses PARP and paracaspase providing a tempting model organism for dissecting the evolutionary conserved cell death pathway. However paracaspase is not required for *D. discoideum* developmental cell death (Uren *et al.*, 2000). Heterologously express caspase-3 (*Sf*-1 cas) in *D. discoideum* using constitutive expression vectors, pA15-cas transfected *D. discoideum* cells did not survive. Evidence suggest that overproduction of the single metacaspase YCA1 in yeast, resulted in autocatalytic processing and rendered cells more sensitive to exogenous or aging-related oxidative stress, as determined by reduced clonogenicity (Madeo *et al.*, 2002). The "induced proximity model" is predicted on the empirical observation that the zymogen forms of unprocessed caspases are not entirely inactive but rather possess weak protease activity. When brought into close contact through protein interactions, the zymogens can trans-process each other, producing the fully active proteases (Wang *et al.*, 2005). In support with this report, our caspase transfectants *D. discoideum* cells might have

induced cell death *via* mechanism similar to induced proximity model of caspase activity.