Regulation of cell survival and death by pyridine nucleotides

Shin-ichi Oka[1,*], **Chiao-Po Hsu**[1,2,*], and **Junichi Sadoshima**[1,3]

[1]Department of Cell Biology and Molecular Medicine, Cardiovascular Research Institute, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, NJ 07103, USA

[2]National Yang-Ming University School of Medicine, Taipei, Taiwan

Abstract

Pyridine nucleotides (PNs), such as NAD(H) and NADP(H), mediate electron transfer in many catabolic and anabolic processes. In general, NAD^+ and $NADP^+$ receive electrons to become NADH and NADPH by coupling with catabolic processes. These electrons are utilized for biologically essential reactions such as ATP production, anabolism and cellular oxidation-reduction (redox) regulation. Thus, in addition to ATP, NADH and NADPH could be defined as high-energy intermediates and "molecular units of currency" in energy transfer. We discuss the significance of PNs as energy/electron transporters and signal transducers, in regulating cell death and/or survival processes. In the first part of this review, we describe the role of NADH and NADPH as electron donors for NADPH oxidases (Noxs), glutathione (GSH) and thioredoxin (Trx) systems in cellular redox regulation. Noxs produce superoxide/hydrogen peroxide yielding oxidative environment, whereas GSH and Trx systems protect against oxidative stress. Then, we describe the role of NAD^+ and NADH as signal transducers through NAD^+-dependent enzymes such as PARP-1 and Sirt1. PARP-1 is activated by damaged DNA in order to repair the DNA, which attenuates energy production through NAD^+ consumption; Sirt1 is activated by an increased NAD^+/NADH ratio in order to facilitate signal transduction for metabolic adaption, as well as stress responses. We conclude that PNs serve as an important interface for distinct cellular responses, including stress response, energy metabolism and cell survival/death.

Keywords

NADPH oxidases; Thioredoxin; Nampt; NAD+; sirtuins; PARP; apoptosis

Pyridine is a basic heterocyclic organic compound with the chemical formula C_5H_5N. PNs are characterized by the presence of a pyridine derivative, such as nicotinamide, as a nitrogen base. For example, nicotinamide adenine dinucleotide (NAD^+) is composed of two riboses and two nucleotides, including nicotinamide and adenine. Nicotinamide and adenine are connected to ribose with glycosidic linkage, and the ribose units bind each other through the phosphate groups (Figure 1A). NAD kinase phosphorylates 2' of the ribose on the adenine side, which generates nicotinamide adenine dinucleotide phosphate ($NADP^+$). Both NAD^+ and $NADP^+$ are subjected to two-electron reduction in the nicotinamide ring to be converted to NADH and NADPH, respectively (Figure 1B).

[3]**Correspondence:** Junichi Sadoshima, MD PhD Cardiovascular Research Institute UMDNJ – Newark 185 S Orange Ave., MSB G609 Newark, NJ 07103 Tel: (973) 972-8619 Fax: (973) 972-8919 sadoshju@umdnj.edu.
[*]These authors contributed equally to this work.

Disclosures
None

The NAD(H) and NADP(H) are involved in many biological reactions as electron carriers. NAD(H) mainly participates in ATP production, while NADP(H) is utilized for modulating cellular reduction and oxidation (redox) status. Increasing lines of evidence suggest that the energy status and the redox status are the major mechanisms regulating survival and death of cardiovascular cell types. For example, the cellular level of remaining ATP is a crucial determinant for the cell to undergo either programmed cell death or necrosis in response to hypoxia. The level of oxidative stress regulated by PNs should affect the extent of cytochrome c release, mitochondrial permeability transition pore opening as well as endoplasmic reticulum stress, thereby serving the signaling mechanism mediating apoptosis and necrosis. Furthermore, NAD+ is used as a substrate for several enzymes, including poly(ADP-ribose) polymereases-1 (PARP-1) and sirtuins, whose targets and end-products critically regulate growth and death of cardiovascular cell types. Thus, the cellular level of PNs significantly affects survival and various forms of cell death through multiple mechanisms.

This review focuses on the roles of PNs in controlling cell death and survival in cardiovascular cells. In the first part, we discuss the role of PNs as electron donors for glutathione (GSH) and thioredoxin (Trx) systems as well as NAD(P)H oxidases (Noxs), key regulators of cellular redox states. In the second part, we discuss regulation of cellular levels of NAD+ and the role of NAD^+ dependent enzymes, such as PARP-1 and sirtuins, which regulate cell survival and death through regulation of their target proteins as well as through consumption of NAD+.

Role of PNs in Cellular Redox Regulation as Electron Carriers

Excessive accumulation of reactive oxygen species (ROS), including superoxide ($O_2^·$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH·), is generally harmful to organisms because they react with cellular components, such as DNA, proteins and lipids. ROS are produced from various sources in cells, such as leakage from mitochondria, metabolic reactions, and irradiations [1]. Oxidative damage of proteins and DNAs often triggers apoptotic cell death, while further production of ROS leads to mPTP opening, ATP depletion and eventual necrotic cell death [2]. Increases in ROS are observed in response to a variety of stress in the heart, including ischemia/reperfusion (I/R) and hemodynamic overload, thereby mediating myocardial injury, hypertrophy and cardiac dysfunction [3]. In order to protect against excessive ROS, cells possess an antioxidant systems, including glutathione (GSH) and thioredoxin (Trx), and reducing enzymes, such as superoxide dismutases and catalase [4].

NAD^+ receives electrons to form NADH by coupling with several catabolic processes, such as the glycolytic pathway, β-oxidation and the TCA cycle. The free energy derived from NADH is utilized for conversion of ADP to ATP in mitochondria via the electron transport chain. $NADP^+$ is converted to NADPH by the pentose phosphate pathway, which is an alternative glycolytic pathway. The electrons derived from NADPH are transferred to the GSH and Trx systems in order to reduce ROS and oxidized proteins. Thus, the cellular antioxidant machineries are maintained with energy provided by glucose catabolism through NADPH-mediated electron transport (Figure 1C). Importantly, however, electrons derived from both NADH and NADPH are also used to generate superoxide and hydrogen peroxide by NAD(P)H oxidases, which in turn stimulates oxidative stress [1]. In the presence of oxidative stress, nitric oxide synthase (NOS), which normally produces NO by transfer of electrons from NADPH to O_2^- and oxidation of L-arginine to L-citrulline, produces superoxide due to uncoupling of the latter process [2]. Mitochondrial electron transport chain in the failing heart produces more free radicals originated from NADH than that in the normal heart [3]. Taken together, PNs play an important role in controlling the cellular redox

status by serving as electron donors for both negative and positive regulators of oxidative stress.

NAD(P)H Oxidases (Noxs)

NADPH oxidases (Noxs) are transmembrane proteins that produce superoxide and/or hydrogen peroxide by transferring electrons provided by either NADH or NADPH to molecular oxygen. Noxs are either activated or upregulated by a variety of extracellular stimuli, including growth factors and cytokines. Thus, ROS production by Noxs is regulated acutely and chronically [6] (Figure 2). This is in contrast to electron leakage from mitochondria, which is a passive mechanism and does not appear to be regulated.

Currently, seven family members of Nox are identified which include Nox(s)1 to 5, Duox1 and Duox2 [1]. Among them, Nox2 and Nox4 are the major enzymes responsible for O_2^- production in CMs (Figure 2). Activation of Nox2 requires stimulus-induced membrane translocation of cytosolic regulatory subunits, including p47^{phox}, p67^{phox}, p40^{phox}, and Rac1, a small GTPase [4]. When cells are stimulated with agonists for G protein-coupled receptors, including the angiotensin II (Ang II) type 1 receptor (AT1-R), Rac1 and a ternary complex of p47^{phox}, p67^{phox}, and p40^{phox} translocate to the membrane, where they form a functional complex with the Nox2-p22^{phox} heterodimer, thereby initiating production of O_2^-[5]. Unlike Nox2, Nox4-mediated O_2^- generation does not require association with cytosolic factors, and Nox4 constitutively generates O_2^-[6, 7].

The superoxide producing activity of Noxs is stimulated by various stresses in a regulated fashion, which in turn leads to cell death in cardiac myocytes. For example, mechanical stress activates Rac1, leading to NADPH oxidase activation in CMs [8]. Angiotensin II (Ang II)-induced CM death is attenuated in p47phox knockout mice[9]. Although the identity of Nox remains to be clarified in these studies, regulation by the cytosolic factors suggests the involvement of the Nox2 isoform. Nox2 is activated by hyperglycemia in CMs, where the generated ROS promote apoptosis through activation of the c-Jun N-terminal kinase (JNK) [10].

Although the activity of Nox4 does not appear to be regulated by the cytosolic factors, expression of Nox4 is upregulated in response to aging, hypertrophic stimuli and heart failure [11]. In neonatal rat CMs, overexpression of Nox4 primarily induces apoptosis, suggesting that the proapoptotic action of Nox4 is cell autonomous [11]. Cardiac-specific overexpression of Nox4 enhances O_2^- production, mitochondrial dysfunction, as well as CM apoptosis in the middle-aged mouse heart *in vivo*[12]. Cardiac-specific Nox4 knockout mice show an ameliorated level of pressure overload-induced CM apoptosis and cardiac dysfunction [13]. Taken together, Nox4-mediated ROS production promotes apoptosis in CMs.

Nox2 and Nox4 exhibit distinct subcellular localizations. Nox2 is expressed primarily on the plasma membrane, whereas Nox4 on intracellular membranes, including those in mitochondria, endoplasmic reticulum and nucleus [12, 13]. Nox4 has a putative mitochondrial localization signal in the N-terminus domain, which is sufficient to direct Nox4 to the mitochondrial membrane [12]. Consistently, oxidation of mitochondrial proteins in response to pressure overload is significantly attenuated in Nox4 KO mice. How Nox4 is inserted in the mitochondrial membrane remains to be shown. In our hands, Nox4 uses NADH more efficiently than NADPH as an electron donor [12, 13]. However, other reports suggest that Nox4 directly generate H_2O_2 through a third cytosolic loop-dependent mechanism [14]. Cardiac responses to pressure overload are quite different between Nox2 (systemic) KO mice and cardiac specific Nox4 KO mice [13, 15]. Thus, ROS produced by Nox2 and Nox4 appear to function differently or in a cell type-dependent manner. The fact that Nox2 and

Nox4 produce ROS at distinct subcellular localizations potentially explains the difference in their phenotype. In addition, Nox2 and Nox4 may produce distinct ROS, namely O_2^- vs H_2O_2, which could also contribute to the distinct cardiac effects between Nox2 and Nox4.

In addition to apoptotic signaling, Noxs are reported to prevent cell death through activation of cell survival signals, especially in cancer cells. Nox2 inhibitors induce cell death in epithelial ovarian cancer [16]. Nox4 also prevents apoptosis, thereby acting as an oncoprotein in breast cancer cells [17] and pancreatic cancer cells [18]. Interestingly, Nox4 can generate O_2^- in low O_2 conditions. O_2^- produced by Nox4 during hypoxia inactivates proline hydroxylase and stabilizes HIF-1α, which in turn mediates cell survival and angiogenesis, both of which are protective against apoptosis. In fact, cardiac dysfunction induced by pressure overload was exacerbated due to insufficient angiogenesis in systemic Nox4 KO mice, suggesting that downregulation of Nox4 below physiological level could be detrimental in some experimental conditions [19], which might represent physiological functions of Noxs and may justify the fact that the heart express an active source of ROS in mitochondria.

GSH

GSH is a small antioxidant protein composed of tripeptide which is L-γ-glutamyl-L-cysteinyl-glycine. GSH exists in reduced (GSH) and oxidized (GSSG) forms. GSH carries electron provided by NADPH and donates a reducing equivalent (H^+ e^-) to reduce proteins with disulfide bonds. GSH is very abundant and exists in the mM order of concentration in cells. GSH is regenerated from GSSG in the presence of GSH reductase. GSH is synthesized through two ATP-dependent steps, namely the first step with glutamylcysteine-synthetase-(γ-GCS) and the second step with GSH synthase. GSH is essential for cell survival, as evidenced by the mortality rate of γ-GCS knockout mice, where the lack of this rate-limiting enzyme for GSH biosynthesis causes massive apoptotic cell death [20]. Overexpression of γ-GCS inhibits TNFα- and UV-induced apoptosis in Hepa1 and HEK293 cells [21, 22]. Increased GSH prevents apoptosis in murine hepatocytes and Cem cells, a leukemic T cell line[23, 24], whereas GSH depletion, using buthionine sulfoximine (BSO), induces or potentiates apoptotic cell death in Cem cells [24]. Interestingly, γ-GCS is a substrate of caspase 3 [25]. The cleavage of γ-GCS may have a role in facilitating the implementation of apoptosis.

The oxidation and reduction cycle between GSH and GSSG further couples with sub-antioxidant systems composed of glutathione-dependent peroxidase (Gpx) and glutaredoxin (Grx) (Figure 1C). These GSH-dependent antioxidant systems have reactive cysteine residues in the catalytic centers and function as carriers of electrons originated from PNs through GSH. Thus far, six family members of Gpx have been identified, namely Gpx1 to 6, and all Gpxs catalyze the reduction of H_2O_2 using electrons provided by GSH [26]. Doxorubicin-induced CM death is exacerbated in Gpx1 knockout mice [27]. Similarly, loss of Gpx2 increases apoptotic cell death in the intestine [28]. Homozygous knockout of Gpx4 in mice results in embryonic lethality at E7.5 [29]. The Gpx4-null cell line is highly sensitive to oxidative stress [29]. Mammalian cells have 4 Grxs including Grx1 to 3 and Grx5 [30]. Grx1 suppresses apoptosis-stimulating kinase 1 (ASK1) activation through direct interaction [31]. In the presence of apoptotic stimuli, Grx1 is oxidized and dissociated from ASK1, thereby activating ASK1-induced apoptosis in MCF7 cells. Grx2 protects against oxidative stress induced by doxorubicin in HeLa cells [32].

Although the fact that excessive oxidative stress imposes many pathological influence in cardiovascular cell types has been established, excessive levels of an antioxidant or excessive reduction, termed reductive stress, has been recently recognized to be also toxic for the heart [33]. αB-crystallin (CryAB) is a small heat shock protein abundantly expressed in CMs. An autosomal dominant missense mutation in CryAB (CryAB (R120G)) causes

cataracts and desmin-related myopathy in humans. CryAB prevents aggregation of proteins inside CMs but proteins improperly unfold and form aggregates in the presence of CryAB(R120G) [34]. In the presence of CryAB(R120G), glucose-6-phosphate dehydrogenase (G6PD), an enzyme producing NADPH, is upregulated, which in turn facilitate a conversion from GSSG to GSH. The enhancement of the cellular reducing power stimulates protein aggregation in CMs, since the reductive environment prevents correct protein folding in the ER [33, 35]. Overexpression of heat shock protein 27 (Hsp27) also increased reductive stress and cardiomyopathy through increases in GSH/GSSG and upregulation of Gpx1[36]. These results suggest that excessive production of the PN and increases in GSH/GSSG are detrimental for the heart. It is unclear, however, how dysregulation of chaperone leads to upregulation of G6PD and increases in GSH/GSSG. In addition, why the increase in NADPH leads to prominent reduction of GSH rather than reduction of Trx1 or induction of O_2^- is unknown. Furthermore, the destination of electrons originated from NADPH and GSH and their roles in mediating cardiomyopathy remains to be elucidated. It will be interesting to investigate posttranslational modification of cysteine residues of cardiac proteins in human desmin-related myopathy.

Trx System

Thioredoxin 1 (Trx1) is a 12KDa protein, which has conserved cysteine residues (Cys-32 and Cys-35) in the catalytic center. These two cysteine residues exist in a reduced state in the presence of NADPH, an electron donor, and Trx reductase1 (TrxR1). Trx1 reduces oxidized proteins by providing an electron. During this process, Trx1 transiently forms an inter-molecular disulfide bond with the target protein by transferring an electron from one of the conserved cysteine residues to the target protein. Trx1 then provides another electron from the other cysteine to reduce the intermolecular disulfide bond. This reaction is termed "thiol disulfide exchange reaction". Importantly, electrons originated from NADPH are first provided to Trx1 and then to the oxidized target protein[37]. Trx1 has specific protein-protein interactions with intracellular signaling molecules and transcription factors. Thus, Trx1 is a carrier of electrons originated from the PN to deliver them to specific targets, which in turn exert a wide variety of function in cells (reviewed in [38, 39]).

Trx1 was originally identified as an electron donor for ribonucleotide reductase which converts RNA to DNA in *E.coli* [37]. Aside from Trx1, Grx1 also acts as an electron donor for ribonucleotide reductase in bacteria [40]. In *E.coli*, the single gene mutant lacking either Trx1 or Grx1 is viable, but the double mutant is not viable in the minimum medium without reduced sulfate [41]. In contrast to *E.coli*, single gene knockout of Trx1 in mice results in early embryonic lethality[42]. Thus, Trx1 is uniquely required for early differentiation and morphogenesis in mammalian cells.

Trx1 plays an important role in protecting against oxidative stress-induced cell death [43]. Since the cellular concentration of GSH is approximately 1-10 mM [44], while that of Trx1 is at μM levels [45], it is unlikely that the protective effect of Trx1 is performed as a general free thiol buffer. Peroxiredoxins (Prxs) have been identified as Trx-dependent peroxidases [46]. Trx1 acts as an electron donor for Prx, which converts H_2O_2 to H_2O. During the identification and characterization of Prxs, it has been demonstrated that Trx1 itself does not effectively scavenge H_2O_2, but rather, Trx1-mediated peroxidase activity is highly dependent upon Prxs [46, 47]. Since H_2O_2 is most abundant among ROS in cells, the insensitivity of Trx1 against H_2O_2 may be important for Trx1 to reduce its specific target proteins. This notion might be also supported by the fact that TrxR is even more resistant against oxidative inactivation compared to Trx1 [48]. Protein oxidation at specific cysteine residues alters the function or activity of target proteins. Trx1-mediated reduction of cysteine residues also exhibits specificity thereby acting as cell signaling mechanisms.

Several transcriptional regulators and other enzymes involved in cardiac hypertrophy and heart failure are directly reduced by Trx1. These include Redox factor-1 [49], NE-F2 related factor (Nrf-2) [50], Nuclear factor-κB (NFκB) [51], p53 [52], FoxO4 [53], Ras [54], methionine sulfoxide reductase [55], and class II histone deacetylases (HDACs) [11]. Importantly, cysteine residues in these molecules are reduced by electrons originated from NADPH through Trx1. Since the major function of Trx1 in the heart is to suppress pathological hypertrophy and apoptosis, identifying the targets of Trx1 may allow us to identify additional key mediators of these pathological events in the heart [56]. The role of Trx1-mediated posttranslational modification in mediating pathological hypertrophy and heart failure has been reviewed recently [38, 39].

Trx1 also mediates anti-apoptotic actions through other mechanisms as well. For example, Trx1 upregulates miroRNA-98/let-7, which in turn suppresses angiotensin II-induced hypertrophy and apoptosis in part though downregulation of cyclin D2 [57]. Trx1 also upregulates genes involved in both mitochondrial oxidative phosphorylation and the TCA cycle, thereby stimulating mitochondrial function in the heart [58]. Trx1 directly interact with ASK1, a pro-apoptotic kinase, thereby inhibiting the kinase activity of ASK1 independently of cysteine modification.

Among Trx1-activating transcriptional factors, posttranslational redox regulation of NF-κB deserves further discussion. All NFκB family proteins possess a conserved motif, namely RXXRXRXXC. Oxidation of the cysteine residue attenuates its DNA-binding ability [59]. Trx1 reduces the cysteine residue and enhances DNA binding of NFκB in the nucleus [51]. In sharp contrast, the upstream signaling pathway leading to NFκB activation is activated by oxidative stress. ROS are produced by proinflammatory cytokines, such as TNFα, which leads to NFκB activation. Overexpression of Trx1 and treatment with N-acetyl cysteine, a plasma-membrane-permeable reducing agent, suppresses TNFκ-induced NFκB activation by inhibition of IKKs activation [60, 61]. Thus, NFκB activation is enhanced by oxidative conditions in the cytosol and by reductive conditions in the nucleus. Interestingly, Trx1 translocates from the cytosol to the nucleus when stimulated by TNFα, Phorbol 12-myristate 13-acetete (PMA) and UVB [62]. Forced expression of Trx1 in the nucleus enhances TNFα-induced NFκB activation [62]. Thus, translocation of Trx1 from the cytosol to the nucleus facilitates NFκB activation by providing an oxidative environment in the cytosol and a reductive environment in the nucleus. Although the mechanism responsible for inhibition of IKK activation by cytosolic-Trx1 has not yet been determined, Trx1 may scavenge H_2O_2 through reduction of Prx1.

Thioredoxin 2 (Trx2)

Oxidative stress in mitochondria plays an important role in mediating mitochondrial dysfunction and cell death during aging and heart failure. Superoxide produced from molecular oxygen at electron transport chain or Nox4 is rapidly dismuted to hydrogen peroxide, whereas mitochondrial hydrogen peroxide is detoxified by the GSH and Trx2 systems. Trx2 is a member of the Trx family and is localized primarily in mitochondria. The GSH and Trx2 systems reduce H_2O_2 through Grxs 1/4 and peroxiredoxin3, respectively. Importantly the reducing activity of the GSH and Trx2 systems is maintained by GSH-reductase and TrxR2, respectively in the presence of NADPH. A recent study showed that energized mitochondria have more reduced Trx2 and that the reducing activity of Trx2 critically depends upon TrxR2 [63].

Loss of Trx2 leads to apoptotic cell death in association with increased ROS levels, cleavage and activation of casepase-3 and caspase-9 in the DT40 cell line [64], suggesting that Trx2 is an essential gene for cell survival by suppressing mitochondrium-dependent cell death. Gene disruption of Trx2 in mice causes massive apoptotic cell death resulting in embryonic

lethality [65]. Cardiac specific deletion of TrxR2 exhibited dilated cardiomyopathy and early lethality [66], suggesting that the TrxR2-Trx2-Prx2 system is critically important in the postnatal heart as well. Suppression of mitochondrial oxidative stress by mitochondrially overexpressed catalase prevents pathological hypertrophy and aging in the heart. Since the level of endogenous catalase is low in mitochondria, intervention to upregulates endogenous mitochondrial Trx system could be an alternative method to prevent pathological events in the heart in elderly patients.

Competition of NADPH/NADH between Nox4 and Mitochondrial Enzyme

Currently, how distribution of electrons from the donor (NADPH/NADH) to the recipients (Noxs, Trx and GSH) is controlled is not well understood. A recent study showed, however, that Nox4 and G6PD are observed in the overlapping microdomains within nucleus and that G6PD controls Nox4 activity through nuclear production of NADPH in the liver, suggesting that G6PD and Nox4 are coupled locally [67]. It is possible that consumption of PNs by Noxs may attenuate regeneration of Trx and GSH, thereby initiating a vicious cycle of oxidative stress, although a recent study showed that overexpression of Nox4 paradoxically increases GSH/GSSG in the heart [68].

The biological toxicity of O_2^- produced by Noxs is due to its capacity to inactivate *the iron-sulfur cluster* containing enzymes (which are critical in a wide variety of metabolic pathways, including the TCA cycle and the mitochondrial electron transport chain) [69], thereby liberating free iron in the cell, which can undergo Fenton chemistry and generate the highly reactive hydroxyl radical. In fact, a series of molecules in the TCA cycle are strongly oxidized in the mitochondrial fraction prepared from aging Nox4 overexpression hearts [12], and pressure overload-induced inhibition of aconitase activity was attenuated in Nox4 KO mice. Since Nox4 preferentially utilizes NADH as an electron donor [7], Nox4 may directly regulate the NADH/$FADH_2$ generating enzymes in the TCA cycle by oxidizing them, thereby initiating regulatory feedback mechanisms controlling their O_2^- producing activity in mitochondria. In addition, consumption of NADH by Nox4 may interfere with electron transport and affect ATP synthesis in mitochondria during heart failure. Further investigation is needed to elucidate the local regulation of mitochondrial enzymes by PNs.

Regulation of Cell Survival and Death by NAD+-Dependent Enzymes

There are enzymes that consume NAD^+, such as poly(ADP-ribose) polymerases (PARPs) and sirtuins. Among PARP family proteins, PARP-1 has a strong impact on NAD^+ consumption. Highly activated PARP-1 leads to depletion of NAD^+ pools in cells. Since NAD^+ is required for ATP production, depletion of NAD^+ attenuates ATP production, resulting in cell death. A major role of PARP-1 is repairing damaged DNA. Whether or not activation of Sirt1, a member of the sirtuin family, has strong impacts on the cellular level of NAD^+ remains to be shown. In general, low nutrition and/or energy deficiency increase NAD^+/NADH ratio where Sirt1 is activated. Sirt1 plays an important role in regulating cell survival and death and metabolic responses to caloric restriction and fasting. Activation of Sirt1 allows cells to alleviate the metabolic stress, and, thus, overconsumption of NAD^+ may not take place. Taken together, PNs regulate cell survival and death by regulating the activity of the NAD^+-dependent enzymes. The level of PNs could be reduced as a result of hyperactivation of the NAD^+-dependent enzymes. The cellular level of NAD^+ affects cell survival and death either by directly affecting the energy status or secondarily affecting other NAD^+-dependent enzymes. In the following, we discuss 1) regulation of NAD^+ in cells, 2) the function of Nampt, a key enzyme regulating the synthesis of NAD^+, and 3) the function of NAD^+-dependent enzymes, including PARP and Sirt1.

Regulation of NAD⁺

In mammals, NAD^+ can be freshly synthesized from amino acids, including tryptophan or aspartic acid, via the *de novo* pathway [70] or taken up efficiently from the extracellular space [71]. Importantly, NAD^+ can also be re-synthesized from NAD^+ metabolites through the salvage pathway [70]. In the salvage pathway of NAD^+ biosynthesis, Nampt was identified as a rate-limiting enzyme that converts nicotinamide into nicotinamide mononucleotide (NMN) [72, 73] (Figure 3). NMN is then directly synthesized into NAD^+ by NMN adenylyltransferase (Nmnat) [74, 75]. Nampt has been paid much attention because it increases the intracellular NAD^+ level efficiently, and it has been proposed as a functional equivalent of pyrazinamidase/nicotinamidase 1 (PNC1), a master gene of longevity in yeast, in mammals. Upregulation of Nampt increases the cellular NAD^+ level and enhances the activity of the catalytic domain of Sirt1 in mouse fibroblasts [73]. It has been shown that there are large differences in the mitochondrial and cytosolic compartmentalization of NAD^+ in different cell types [76]. This implies that NAD^+ concentration could be different among different compartments in the cell[76]. An isoform of Nmnat, termed NMNAT3, is enriched in mitochondria [77], suggesting that production of NAD^+ may be regulated in a subcellular compartment.

Nampt

The gene that encodes for Nampt/PBEF was isolated from a human peripheral blood lymphocyte cDNA library termed pre-B-cell colony-enhancing factor (PBEF), because it was thought to be a cytokine that enhances the effect of interleukin-7 (IL-7) and stem cell factors (SCFs) on pre-B-cell colony formation [78]. Later, on the basis of significant homology between PBEF and nadV, a prokaryotic nicotinamide phosphoribosyltranferase (NAmPRTase) from *Haemophilus ducreyi*, PBEF was identified as Nampt, an enzyme involved in NAD^+ biosynthesis [72, 79]. Visfatin, one of the adipocytokines, was also found to be an identical molecule as Nampt. Although it was originally proposed that Visfatin has insulin-mimetic effects in cultured cells, Nampt was found later to be a regulator of insulin secretion, rather than an insulin-mimetic, in pancreatic β-cells [80]. Nampt is a highly conserved 52-kDa protein with no signal sequence, but it exists in most cells and tissues [78]. In addition to its major role in intracellular NAD^+ biosynthesis, Nampt also acts as an extracellular NAD^+ biosynthetic enzyme whose secretion is observed in neutrophils, adipocytes and mesangial cells[80].

Nampt is upregulated in neutrophils in response to a variety of inflammatory stimuli [81] in smooth muscle cells (SMCs) during maturation, and it lessens SMC apoptosis [82]. Recently, Nampt was found to be upregulated in primary neonatal rat CMs under specific stresses (*in vitro*) and nutrient restriction (*in vitro*), and it was even upregulated in the liver tissue of fasting Sprague-Dawley rats [83]. Nampt was also shown to protect against necrosis and apoptosis through mitochondrial NAD^+-dependent deacetylases Sirt3 and Sirt4 [83]. Moreover, PARP expression and NAD^+ consumption were significantly increased in light of the genetic instability in tumors [84], and Nampt was shown to be upregulated in colorectal cancer [85]. These findings suggest that Nampt is a stress-responsive protein that protects cells against injury and promotes cell survival. However, expression of Nampt in the heart was downregulated in response to some pathological stimuli in our study (*in vivo*) [86], and Dahl et al. also noted a decrease in the serum levels and hepatic mRNA levels of Nampt in nonalcoholic fatty liver disease [87]. Hence, the regulation of Nampt is stimulus- as well as tissue-specific.

The NAD^+ salvage pathway was recently found to be involved in circadian rhythm regulation through epigenetic control and chromatin remodeling [88, 89]. The percent change of intracellular NAD^+ concentration in liver tissue has a maximum of only 50%; however, it

can accurately regulate the activity of Sirt1 in the circadian cycle. Furthermore, the transcription of Nampt may be modulated by the Sirt1-Clock:Bmal1 via a feedback loop. These pieces of evidence point out that the intracellular coupling of Nampt and Sirt1 must be intimate even without direct physical interaction.

Regulation of Oxidative Stress—In the human aortic endothelial cell (HAEC), Nampt can reduce cellular accumulation of ROS, an important cause of senescence, aging and cell death, in response to low and high glucose at least partly through sirtuin-dependent mechanisms. Overexpression of Nampt enhances proliferation, replicative lifespan and genomic stability in HAEC [90]. Expression and activity of Nampt declined with senescence of human SMCs where Nampt protects against oxidative stress via enhanced Sirt1 activity and p53 degradation [91]. Cardiac-specific overexpression of Nampt in transgenic mice increases NAD^+ content in the heart reduces the size of myocardial infarction and apoptosis in response to prolonged ischemia and ischemia/reperfusion, which evoked a great impact on oxidative stress [86]. Genetic disruption of angiotensin II type1 receptor (AT1R) promotes prolongation of lifespan in mice *in vivo*. The heart, aorta and kidneys from $AT1R^{-/-}$ mice displayed less oxidative damage than wild-type mice, which was associated with an increased number of mitochondria and upregulation of Nampt and Sirt3 in the kidney [92]. Nampt can sustain intracellular NAD^+ availability, even in mitochondria, through more efficient NAD^+ production, when stresses induce consumption of NAD^+ and ATP. It should be noted that there are some exceptions. Overexpression of Nampt increases its interactions with NADH dehydrogenase subunit 1, ferritin light chain, and interferon induced transmembrane 3, each of which are involved in oxidative stress and inflammation. Consequently, intracellular oxidative stress in human pulmonary vascular endothelial cells is increased, which may convey a mechanism of Nampt in the pathogenesis of acute lung injury [93]. Regardless of whether it can universally decrease oxidative stress or not, Nampt indeed modulates oxidative activity.

Metabolic Effects—Nampt and NAD^+ regulate the activity of sirtuins [73]. Sirt1 can deacetylate many targets, thereby participating in a wide array of cellular processes [94]. Some of its targets have metabolic effects, including PGC-1α, the FOXO family of transcription factors and acetyl-CoA synthetase 1 (AceCS1). Therefore, it is logically assumed that Nampt can modulate metabolic effects through the activity of Sirt1.

Overexpression of Nampt elicits a modest increase in aerobic glycolysis in response to high glucose (30mM) media, which could be blocked in the presence of either FK-866, a chemical inhibitor for Nampt, or sirtinol, an inhibitor for Sirt1,but it does not affect lactate production, glucose transport and oxidation in HAEC [90]. Fatty acid β-oxidation was marginally increased in the low glucose medium (5mM). This data suggests a subtle but significant preference for ATP generation through aerobic glycolysis in Nampt-overexpressing HAEC, which may require Sirt1 activity. These findings are consistent with those on the effects of Sirt1, such as the increase of fatty acid oxidation in response to low glucose concentrations in skeletal muscles [95], and the inhibition of fat storage and the triggering of lipolysis in fat cells through suppression of the nuclear receptor PPAR-γ during calorie restriction [96].

The detrimental effect of elevated free fatty acids (FFAs) on insulin sensitivity could be normalized by thiazolidinediones (TZDs) via increased secretion of Nampt in patients with type 2 diabetes mellitus. Nampt secretion was blocked by synthetic fatty acids and by inhibition of phosphatidylinositol 3-kinase (PI3K) or Akt in human adipocytes [97]. Nampt could regulate insulin secretion in β-cells through Nampt-mediated systemic NAD^+ biosynthesis, suggesting vital roles for Nampt and NAD^+ in the regulation of glucose metabolism [80]. The synthesis and secretion of Nampt could be increased by a high level of

glucose stimuli in cultured mesangial cells (MCs). Nampt treatment induces a rapid uptake of glucose via increased glucose transporter-1 (GLUT-1) protein expression in cellular membranes, which could be blocked by siRNA against insulin receptors and FK866 [98]. Furthermore, glucose restriction inhibits skeletal myoblast differentiation via the activated AMPK-Nampt-Sirt1 pathway [99]. PPARα activation and glucose downregulated hepatic Nampt expression *in vitro* and *in vivo* [87]. Nampt could be regarded as a sensor of intracellular energetic conditions in response to nutritional availability. These pieces of metabolic evidence have provided a possible molecular pathway connecting Nampt to cell survival and life extension.

Protection against Cell Death—Nampt is induced and secreted by peripheral blood mononuclear cells in response to inflammatory stimuli (LPS, IL-1β and TNF-α). Nampt inhibits apoptotic cell death in neutrophils, which was associated with reduced activity of caspases-8 and -3, but not of caspase-9. This indicates that the death receptor pathway was attenuated [81]. Nampt protects against cell death (including necrosis and apoptosis) through mitochondrial NAD^+-dependent deacetylases Sirt3 and Sirt4 [83]. Enzymatic activity of Nampt is required for its resistance to oxidative (genotoxic) stress where Nampt prevents apoptosis-inducing factor (AIF) delocalization from the mitochondrial innermembrane space in response to methyl-N'-nitro-N'-nitrosoguanidine, which is a PARP-1-dependent (caspase-independent) cell death. Nampt may modulate cell viability by affecting an earlier event preceding AIF release [100]. Intracellular Nampt inhibited Huh7 hepatocytes apoptosis through NAD^+ synthesis [87]. However, we have shown that Nampt positively regulates survival and that downregulation of Nampt induces the release of cytochrome *c* from mitochondrial intermembrane space to the cytosol in cardiac myocytes, suggesting that the mitochondrial component of apoptosis is involved in cell death induced by Nampt downregulation [86]. We also showed that Sirt1 downregulation could cause apoptotic cell death in CMs [101]. Furthermore, there is no additive effect on increases in TUNEL-positive myocytes after downregulation of both Nampt and Sirt1, suggesting that downregulation of Nampt may induce cardiac myocyte apoptosis via a common mechanism such as downregulation of Sirt1 [86]. As a whole, this implies that the effect of Nampt inhibition on the mode of cell death is stimulus-specific and cell type-dependent, and that NAD^+ plays an important role in controlling cell survival.

Not all anti-apoptotic effects of Nampt can be explained by NAD^+ biosynthetic activity. For example, NMN, but not Nampt, protected macrophages from ER stress-induced apoptosis by activating an interlukin-6(IL-6)/STAT3 signaling pathway, indicating that the activation does not require Nampt enzymatic activity. Rather, it includes a two-step sequential process: rapid induction of IL-6 secretion, followed by IL-6-mediated activation of the prosurvival signal transducer STAT3 [102]. However, the mechanism by which NMN triggers IL-6 secretion is currently unknown.

Stimulation of Autophagy—Autophagy, an evolutionarily conserved process for the bulk degradation of cytoplasmic components, not only serves as a cell survival mechanism in starving cells but also mediates autophagic cell death in some conditions, such as I/R [103]. Billington et al. showed that the autophagic mechanism could be modulated by NAD^+[71]. Recently, we found that downregulation of Nampt inhibited autophagic flux in rat neonatal cardiac myocytes, which was reversed by the exogenous application of NAD^+. Meanwhile, there were no additive effects on autophagic flux after downregulation of both Nampt and Sirt1 [86]. It was proposed that Nampt may affect the level of NADH, an reducing equivalent in cells, which in turn affects the activity of the electron transport chain on the lysosomal membrane. If the function of the electron transport chain is inhibited, lysosomal acidification and autophagosome-lysosome fusion as well as lysosomal degradation may be

impaired [104]. This implies that the regulation of autophagy by Nampt is mediated through NAD+ and/or Sirt1 (See below).

Angiogenesis—Xenotransplantation of Nampt-overexpressing SMCs into immunodeficient mice displayed an increased capacity for the transplanted SMCs to mature and intimately invest nascent endothelial channels [82], suggesting that Nampt stimulates angiogenesis. Hypoxia induces Nampt expression through hypoxia-inducible factor-1α in MCF7 breast cancer cells [105]. Nampt promotes migration, invasion and tube formation in human endothelial cells via activation of extracellular signal-regulated kinase 1/2-dependent FGF-2 expression [106] and/or STAT3-dependent endothelial IL-6 induction [107]. Nampt induces angiogenesis through endothelial VEGF and MMP2/9 production [108]. Furthermore, MCP-1 is pivotal in modulating Nampt-induced angiogenesis via NF-κB and PI3K pathways, and autocrine/paracrine mechanisms (via the CCR2 receptor) [109]. Augmenting Nampt activity enhances angiogenic response via Sirt1-mediated repression of FoxO1 in HAEC [90]. Taken together, these results demonstrate that Nampt promotes angiogenesis, an important mechanism for cell survival.

Clinical Implication—Nampt/NAD+ promotes cell survival not only in normal cells, but also in cancer cells. FK866 effectively induces apoptotic cell death by gradual depletion of the intracellular NAD+ level in tumor cells (HepG2) [110]. A strong anti-angiogenic potency in FK-866 was seen in a murine renal cell carcinoma model [111]. Thus, it is in theory reasonable to inhibit Nampt to potentiate the chemotherapeutic effect of other antineoplastic agents.[112]. CHS-828 (a pyridyl cyanoguanidines), a potent chemoagent, also kills cancer cells by depleting NAD+ via inhibition of Nampt function and NF-κB signaling [113]. Both of those drugs are under evaluation in clinical trials for cancer treatment.

Nampt is upregulated and promotes survival of neutrophils during immune and inflammatory responses in both human and animal models [81], and it can induce leukocyte adhesion to endothelial cells and the aortic endothelium through induction of cell adhesion molecules, including ICAM-1 and VCAM-1 [114]. Proteinuria, which is an important predictor of endothelial dysfunction in early diabetic nephropathy, was associated with altered circulating levels of Nampt and adiponectin [115]. Nampt could be a novel marker for endothelial damage in renal transplantation [116]. Nampt can be regarded as a proinflammatory molecule, and its inhibitor, FK866, can effectively reduce the severity of symptoms in mice with collagen induced arthritis [117]. Macrophage plays a key role in obesity-associated pathophysiology [118]. Several lines of evidence support a positive correlation of serum or plasma Nampt with obesity and diabetes [119, 120], suggesting a potentially important role of Nampt in the pathogenesis of obesity and type 2 diabetes.

PARP-1

PARP-1 is an abundant nuclear protein, which polymerizes ADP-ribose on the target proteins, a reaction termed poly ADP-ribosylation (Figure 4) [121]. The ADP-ribose units are derived from NAD+, and therefore, PARP-1 consumes NAD+. The enzymatic reaction also produces nicotinamide, a natural inhibitor for the PARP-1 and sirtuin families. Poly ADP ribose (PAR) polymers are attached to glutamate, aspartate or lysine residues on target proteins. PAR is negatively charged and acts as a posttranslational modifier. PARP-1 also produces free PAR polymers, which themselves act as a signal transducer. The significance of PARP-1 has been demonstrated in many cellular processes including DNA repair, genomic stability, cell death and survival, and transcriptional regulation. PARP-1 facilitates these functions through poly ADP-ribosylation, free PAR polymer formation, byproduct, protein-protein interaction and depletion of NAD+.

The Role of PARP-1 in Base Excision Repair (BER)—DNA damage is caused by multiple mechanisms, including oxidative damage and alkylation damage. DNA damage induces irregular pairing, which could eventually cause a mutation through incorporation of an incorrect nucleotide. The damaged DNA is repaired by a process termed base excision repair (BER) (Figure 5). In the first step of BER, damaged bases are recognized by DNA glycosylase and are removed by cleavage of an N-glycosidic bond. Next, Apurinic/apyrimidinic (AP) endonuclease cleaves the DNA backbone, thereby generating a single-strand DNA nick. PARP-1 recognizes the DNA nick as a single-strand break and facilitates poly ADP-ribosylation of target proteins. Foci of PAR can be detected within several minutes after hydrogen peroxide treatment or single-stranded DNA break induction [122]. The PAR recruits X-ray repair cross-complementing 1 (XRCC1), an important scaffold protein for base excision repair. PARP-1 is subjected to self- poly ADP-ribosylation and binds to XRCC1 at the site of the single-strand DNA break [122]. XRCC1 interacts with DNA polymerase II (polB) that fills the gap, and DNA ligase III that completes the repair process. PARP-1 knockout cells are highly sensitive to alkylating agents, toposiomerase inhibitors and γ-ray irradiation [123], consistent with the notion that PARP-1 plays an important role in mediating DNA repair. Double-strand DNA breaks are repaired by two distinct pathways: error-prone non homologous end-joining (NHEJ) and error-free homologous recombination (HR). The NHEJ process is initiated by the binding of the Ku70/80 heterodimer and DNA-activated protein kinase to the double-strand break end. Ku70 and Ku80 are subjected to PAR by PARP-1. The PAR decreases the affinity of Ku to the double-strand DNA break end, thereby inhibiting the NHEJ repair process [124]. Thus, PARP-1 promotes HR by inhibition of NHEJ [125], since NHEJ and HR directly compete with each other [126].

PARP-1 Cleavage by Caspase-3—PARP-1 is a caspase-3 substrate. The caspase-dependent cleavage releases the N-terminal DNA binding domain from PARP-1 [127]. Since PARP-1 activity is highly dependent on DNA binding, the cleaved N-terminal fragment competitively inhibits the function of endogenous PARP-1 competing DNA binding with uncleaved PARP-1 [128]. The proteolytic inactivation of PARP-1 is achieved not only by breaking down intact PARP-1 to a non-functional form, but also by dominant negative inhibition by the cleaved product. Since PARP-1-mediated DNA repair is a crucial process for cell survival, the proteolytic inhibition of PARP-1 is likely important for ensuring apoptotic cell death. Indeed, PARP-1-induced BER is blocked by the N-terminal DNA-binding fragment of PARP-1 [128]. The PARP-1 inhibition is also important for preventing hyperactivation of PARP-1, which would otherwise result in depletion of cellular NAD$^+$ as well as ATP. Apoptosis cannot be effectuated when cellular ATP levels are depleted, which results in necrosis [129]. A specific DNase (CAD, caspase-activated DNase) that cleaves chromosomal DNA makes a complex with ICAD (inhibitor of CAD), which works as a specific chaperone for CAD. Caspase 3-cleaves ICAD and allows CAD to dissociate from ICAD and cleave chromosomal DNA. Caspase-mediated cleavage of PARP-1 may prevent hyperactivation of PARP-1 by fragmented DNA, which would induce necrosis. Indeed, the PARP-1 mutant, which cannot be cleaved by caspase-3, promotes necrotic cell death by TNF-α treatment [130].

Parthanatos—In response to low levels of genotoxic stress, PARP-1 promotes cell survival partly through DNA repair, while severe DNA damage triggers PARP-1 hyperactivation to induce cell death. At least two distinct mechanisms are proposed for PARP-1-induced cell death. Firstly, hyperactivation of PARP-1 results in ATP depletion, causing cells to undergo necrotic cell death. Secondly, PAR generation, stimulates AIF dependent programmed cell death, termed parthanatos (Figure 6). Parthanatos is induced by N-methl-D-asparate (NMDA) in neurons and is distinct from apoptosis and necrosis [131]. Different from apoptosis, parthonatos does not form apoptotic bodies or induce small DNA

fragmentation. The PARP-1-induced parthanatos cannot be prevented by pancaspase inhibitors, such as z-VAD-fmk, suggesting that parthanatos is a caspase-independent cell death program [132]. Since hyperactivation of PARP-1 induces NAD^+ depletion, PARP-1-induced cell death was presumed to result from intracellular energy depletion [129]. However, PARP-1-induced cell death can take place even with a minor effect upon ATP content in some cases [133]and, thus, ATP depletion may not be necessary for PARP-1-induced cell death. Instead, the essential role of PAR produced by PARP-1 has been proposed as the mechanism of cell death [131]. The NMDA-induced cell death is attenuated by a neutralization antibody against PAR and by overexpression of PAR glycohydrolase, an enzyme that breaks PAR down. Exogenous PAR injection triggers AIF-dependent cell death. Thus, PAR itself is sufficient to induce cell death.

The PAR generated by PARP-1 translocates from the nucleus to the cytosol. The PAR induces release of the AIF from mitochondria. The essential role of AIF in PARP-1-induced parthanatos has been proposed [132]. Either knockdown of AIF or treatment of AIF neutralization antibody blocks PARP-1-induced cell death. Although a precise mechanism remains to be elucidated, direct interaction of PAR and mitochondria facilitates AIF release. Then, AIF translocates to the nucleus to promote DNA fragmentation; this fragmented DNA size is approximately 50 kbp, which is distinct from the small size of DNA fragmentation in apoptotic cell death. It remains unknown how nuclear AIF induces this large-scale DNA fragmentation.

Although PARP-1-mediated DNA repair is important for cell survival, NAD^+ depletion and PAR promote either necrotic cell death or parthanatos. Whether PARP-1 induces cell survival or distinct forms of cell death depends upon the type of stress, the intensity of stress or the level of NAD+ consumption.

In the heart, pressure overload and I/R activates PARP-1 possibly through oxidative stress-induced DNA damage, which in turn induces translocation of AIF from mitochondria to the nucleus [134, 135] and consequent cell death. A PARP-1 inhibitor reduces AIF translocation, infarct size and cardiac dysfunction after I/R [134]. Genetic deletion of PARP-1 in mice ameliorates I/R-induced cardiac and mitochondrial dysfunction [136]. Downregulation of PARP-1 also prevents AIF translocation, cardiac hypertrophy and dysfunction in response to pressure overload [135]. The beneficial effects of pharmacological and genetic inhibition of PARP-1 are also observed during cardiac dysfunction induced by septic shock [137], angiotensin II-induced cardiac hypertrophy and fibrosis [138], and doxorubicin-induced myocyte death and cardiac dysfunction [139], Interestingly, the beneficial effect of the ischemic preconditioning is abolished in PARP-1 knockout mice [140], suggesting that PARP-1 is protective in some experimental conditions. The function of PARP-1 in the heart critically depends upon the strength of the stress. For example, in the presence of mild to moderate stress, PARP-1 facilitates DNA repair to prevent further DNA damage and accumulation of mutations. At high levels of stress, cells undergo apoptotic cell death, where PARP-1 is subjected to proteolytic inactivation to restore NAD^+ and ATP for effectuating apoptotic cell death. In the presence of more severe stress, hyperactivation of PAPR-1 facilitates necrotic cell death or parthanatos through depletion of NAD^+/ATP.

Sirtuins

Since the function of sirtuins is discussed separately in this review series, here we discussed only briefly on their role in mediating cell survival/death. Sirt1 is an NAD^+-dependent histone and protein deacetylase [141]; an acetyl-group of target proteins is removed and conjugated to the ADP-ribose unit derived from NAD^+. Nicotinamide is also produced as a byproduct in this enzymatic reaction (Figure 7). NADH acts as a competitive inhibitor of Sirt1, and therefore, an increased NAD^+/NADH ratio is critical for Sirt1 activation [142]. In

mammalian cells, the NAD$^+$/NADH ratio is increased by caloric restriction in several organs, which is associated with Sirt1 upregulation and activation [143]. Sirtuin is also inhibited by nicotinamide, an NAD$^+$ catabolite. Sirt1 is involved in a variety of biological reactions, including lipid and glucose metabolism, development, cell growth, immune reaction, autophagy, apoptosis and cancer development.

Regulation of Apoptosis by Sirt1—In general, Sirt1 acts as an anti-apoptotic factor. Expression of Sirt1 is increased in both in vitro and in vivo model of cardiac hypertrophy [144]. Sirt1 inhibition enhances cell death in cultured myocytes [144]. Sirt1 is a negative regulator of the tumor suppressor protein p53 which induces apoptotic cell death in response to genotoxic stress. Acetylation of p53 leads to stabilization and activation of p53, which triggers apoptotic cell death and cell cycle arrest. Sirt1 deacetylates p53 thereby attenuating p53-mediated apoptotic cell death [145, 146]. Sirt1 knockout in mice enhances radiation-induced acetylation of p53 in thymocytes [147]. Interestingly, the Sirt1 knockout mice show developmental abnormalities in the heart, including septal and valvular defects [147]. Cardiac-specific overexpression of Sirt1 prevents age-induced CM death in association with downregulation of p53 [144]. Sirt1 also regulates FoxO transcription factors, which involve in energy metabolism and cellular stress responses [148, 149]. In response to oxidative stress and I/R, Sirt1 deacetylates FoxOs, which leads to nuclear translocation of FoxOs [86, 148]. The nuclear FoxOs play an important role in resistance against ROS as well as apoptotic cell death. Cardiac-specific knockout of Sirt1 enhances apoptotic cell death in ischemia-reperfusion injury [150]. The Sirt1 knockout mice show increased expression of proapoptotic proteins, such as Bax, decreased expression of anti-apoptotic proteins such as Bcl-xL, and decreased expression of anti-oxidants, such as Trx1 and MnSOD [150]. Sirt1 promotes cell survival in the failing heart through upregulation of MnSOD[151]. Sirt1 also mediates ischemic preconditioning through lysine deacetylation of target proteins, such as p53, in the isolated perfused heart preparations [152]. Sirt1 mediates cell survival through both transcriptional and posttranslational mechanisms.

Regulation of Autophagy by Sirt1—Increasing lines of evidence suggest that Sirt1 stimulates autophagy at baseline and in response to stress [153, 154]. Perhaps, energy starvation increases NAD$^+$/NADH, which in turn stimulates sirtuins, which could serve as a sensor for the energy status in cells. Glucose deprivation-induced autophagy is inhibited by knockdown of Sirt1, whereas overexpression of Sirt1 is sufficient to induce autophagy [155]. The Sirt1-induced autophagy is mediated by FoxO1 [155]. Under glucose deprivation, FoxO1 is deacetylated and activated by Sirt1. A critical target gene of FoxO1 includes Rab7, which is a GTP-binding protein that promotes autophagosome-lysosomal fusion [154]. Cardiac specific knockout of FoxO1 shows impaired cardiac function under starvation conditions, suggesting that Sirt1/FoxO1-mediated autophagy plays an important role in maintaining cardiac function under starvation conditions [154]. It is interesting to note that Sirt1, FoxO and autophagy are all involved in lifespan extension and stress resistance in lower organisms. Together with the fact that Nampt also regulates autophagy in the heart through an NAD+-Sirt1-dependent mechanisms suggest that the PN-regulated signaling pathway is a fundamentally important mechanism controlling survival and death of cardiomyocytes and that autophagy takes part of it.

Antagonizing Effect between PARP-1 and Sirt1

PARP-1 and Sirt1 functionally antagonize one another. Both enzymes generate nicotinamide, a natural inhibitor for both Sirt1 and PARP-1. In response to DNA damage, PARP-1 localizes to the site of DNA damage, which leads to local PAR production, NAD$^+$ depletion and nicotinamide generation. The local NAD$^+$ depletion and nicotinamide generation induces histone acetylation through Sirt1 inhibition, which results in chromatin

decondensation [156]. This decondensated chromatin structure may be important for recruiting the DNA repair machinery and facilitating the repair process. In the failing heart, hyperactivation of PARP-1, is associated with NAD+ depletion and Sirt1 inactivation [138]. Sirt1 function is enhanced when PARP-1 is inhibited. PARP-1 knockout mice show increased mitochondrial function, oxygen consumption and energy expenditure in a Sirt1-dependent manner [157].

On the other hand, Sirt1 activation results in reduced PARP-1 activity, whereas hydrogen peroxide-induced PARP-1 activation is enhanced in Sirt1 knockout cells [158]. Loss of Sirt1 potentiates hydrogen peroxide-induced AIF nuclear translocation and cell death [158]. Sirt1 directly inhibits PARP-1 through PARP-1 deacetylation [159]. Acetylated PARP-1 shows high enzymatic activity independently of DNA damage. Sirt1 inhibits PARP-1-induced cell death through direct deacetylation of PARP-1 [159]. Sirt1 also attenuates PARP-1 promoter activity [159] and downregulates PARP-1 expression. In contrast to PARP-1's localization to active promoters, combined analyses of ChIP on ChIP and gene expression profile revealed that Sirt1 mainly localizes on inactive promoters [160]. During hydrogen peroxide–induced DNA damage, PARP-1 interacts with chromatin whereas Sirt1 is released from chromatin [160]. Such reciprocal interaction with chromatin between PARP-1 and Sirt1 may have a role in the DNA repair process and gene transcription. These results demonstrate a crucial link between Sirt1 and PARP-1 in stress response.

Conclusions

In summary, PNs are high-energy intermediates and crucial cofactors that protect against multiple stresses such as ROS and starvation, in many cardiovascular cell types (Figure 8). The functions of PNs are mediated primarily through the GSH and Trx systems, Noxs, Sirt1 and PARP1. During the past decade, a dramatic advancement was seen in the elucidation of the cellular functions of these PN-dependent molecules. As we summarized in this review, all of these molecules clearly play important roles in controlling cellular survival and death in cardiovascular cell types through regulation of cellular redox, energy metabolism and stress responses. However, there are still many outstanding questions. For example, the local coupling mechanism between NADPH/NADH and redox enzymes, namely GSH and Trx1/2, and that between Nampt and NAD^+-dependent enzymes, including PARP-1 and sirtuins, remain to be elucidated. Although the source of PN production and the PN-dependent enzymes appear to be localized closely in subcellular compartments, essentially nothing is known regarding how they are coupled functionally in both physiological and pathological conditions. For example, all electrons utilized by the Trx and GSH systems are derived primarily from the pentose phosphate pathway via NADPH at physiological conditions. However, the source of PNs may be altered during pathological conditions. In the same token, more studies are needed to clarify how the activity of one PN-dependent enzyme affects the other PN-dependent ones. Whether activation Noxs could affect the other PNs-dependent process, including reduction of GSH/Trx and ATP synthesis through the mitochondrial electron transport chain remains to be clarified. More studies are needed to clarify the functional interaction between PARP-1 and Sirt1 in the heart since both PARP-1 and Sirt1profoundly affect cardiac function in the failing heart. Finally, more studies are needed to elucidate the functional significance of NAD^+ production by Nampt in various cell types, subcellular compartments, as well as extracellular spaces, and the function of the sirtuin family members, using genetically altered mouse models. It is likely that such studies should allow us to identify a novel function of PNs in cardiovascular cell types.

Acknowledgments

List of abbreviations

AceCS1	Acetyl-CoA synthetase 1
AIF	Apoptosis-inducing factor
AMPK	5'adenosine monophosphate-activated protein kinase
Ang II	Angiotensin II
AP	Apurinic/apyrimidinic
ASK1	Apoptosis signal regulating kinase 1
AT1R	Ang II type 1 receptor
Bcl-xL	B-cell lymphoma-extra large
BER	Base excision repair
BMAL1	brain and muscle aryl hydrocarbon receptor nuclear translocator (ARNT)-like 1
BSO	Buthionine sulfoximine
CAD	Caspase-activated DNase
CCR2	Chemokine (C-C motif) receptor 2
ChIP	Chromatin immunoprecipitation
CM	Cardiomyocytes
CryAB	αB-crystallin
ER	Endoplasmic reticulum
FAD	Flavin adenine dinucleotide
FGF	Fibroblast growth factor
FoxO	Forkhead box protein O
γ-GCS	γ-Glutamylcysteine-synthetase
GLUT-1	Glucose transporter-1
GSH	Glutathione
GSSG	Glutathione disulfide
Gpx	Glutathione dependent peroxidase
Grx	Glutaredoxin
G6PD	Glucose-6-phosphate dehydrogenase
HAEC	Human aortic endothelial cell
HDAC	Histone deacetylase
HR	Homologous recombination
Hsp	Heat shock protein
ICAM-1	Inter-Cellular Adhesion Molecule 1

IKK	IκB kinase
IL	Interleukin
IR	Ischemia/reperfusion
JNK	c-Jun N-terminal kinase
KO	Knockout
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MC	Mesangial cell
MCP-1	Monocyte chemotactic protein-1
MMP	Matrix metalloproteinase
MnSOD	Manganase superoxide dismutase
mPTP	Mitochondrial permeability transition pore
NAmPRTase	Nicotinamide phosphoribosyltranferase
Nampt	Nicotinamide phosphoribosyltransferase
NFκB	Nuclear factor-κB
NHEJ	Non homologous end-joining
NMDA	N-methl-D-asparate
NMN	Nicotinamide mononucleotide
NMNAT	Nicotinamide mononucleotide adenylyltransferase
NO	Nitrogen oxide
Nox	NADPH oxidase
PAR	Poly ADP ribose
PARP-1	Poly [ADP-ribose] polymerase 1
PBEF	Pre-B-cell colony-enhancing factor
PGC-1α	PPAR-gamma coactivator-1α
Phox	Phagocytic oxidase
PI3K	Phosphatidylinositol 3-kinase
PMA	Phorbol 12-myristate 13-acetete
PN	Pyridine nucleotide
PNC1	Pyrazinamidase/nicotinamidase 1
PPAR	Peroxisome proliferator activated receptor
Prx	Peroxiredoxin
Rab7	Ras(Rat sarcoma)-related in brain
ROS	Reactive oxygen species
Sirt1	Silent information regulator 1
SMC	Smooth muscle cell

STAT	Signal transducer and activator of transcription
TCA	Tricarboxylic acid
TNFα	Tumor necrosis factor-α
Trx	Thioredoxin
TrxR	Thioredoxin reductase
VCAM-1	Vascular cell adhesion protein 1
VEGF	Vascular endothelial growth factor
UV	Ultra violet
XRCC1	X-ray repair cross-complementing 1

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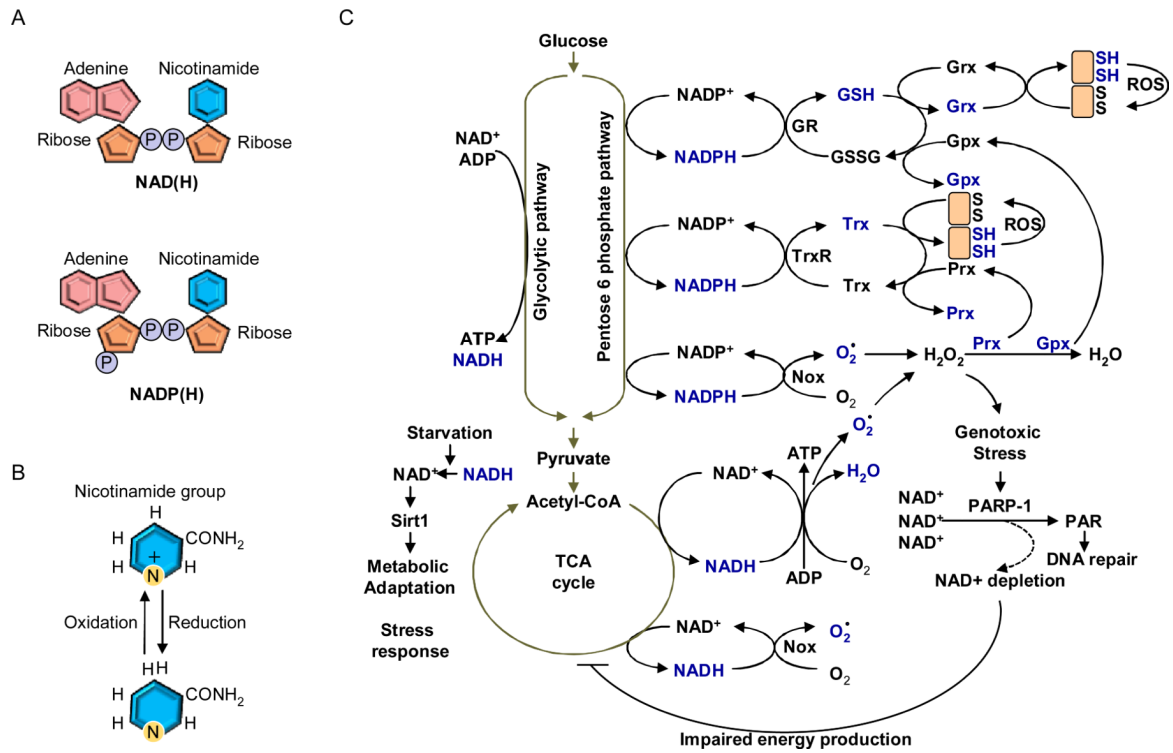
Figure 1.
(A) Schematic structures of NAD(H) and NADP(H). Adenine and nicotinamide connect to ribose unit. Those riboses connect each other through pyrophosphate. NADP(H) has a third phosphate group connected to the ribose unit of adenosine. (B) Oxidized and reduced forms of nicotinamide group. Nicotinamide group of NAD^+ and $NADP^+$ acquires two electrons to convert to NADH and NADPH, respectively. (C) Overview of the biological roles of PNs as electron carriers and signal mediators. NADP+ is reduced by the pentose phosphate pathway. The NADPH is utilized for scavenging ROS as well as reducing oxidized proteins through Trx and GSH systems. NAD^+ is reduced by several catabolic pathways such as the glycolytic pathway and the tricarboxylic acid (TCA) cycle. The electron provided from NADH is transferred to the electron transport chain to convert ADP into ATP. Noxs utilize both NADH and NADPH as electron donors. Genotoxic stress including ROS, damages DNA, which is recognized by PARP-1. The highly activated PARP-1 facilitates poly ADP-ribosylation, an important step in DNA repair, resulting in depletion of NAD^+. Energy production is impaired when NAD^+ is depleted. Under starvation conditions, nutritional catabolism is attenuated, which results in an increased NAD^+/NADH ratio resulting in activation of Sirt1. Grx: Glutharedoxin; Gpx: Gluthathione perodixdase; Prx: Peroxiredoxin.
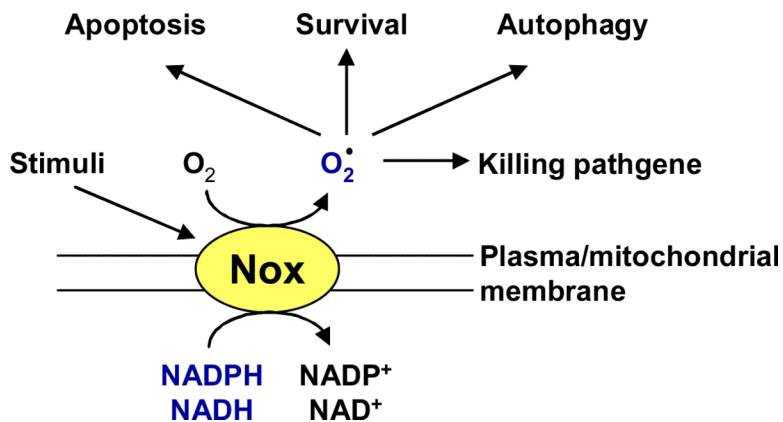
Figure 2.
Nox is activated by a wide variety of stimuli. The electrons derived from NADPH or NADH are transferred across the membrane to generate superoxide or hydrogen peroxide. The Nox derived ROS induce posttranslational modification of mitochondrial proteins and signaling molecules involved in cell survival, apoptosis or autophagy.
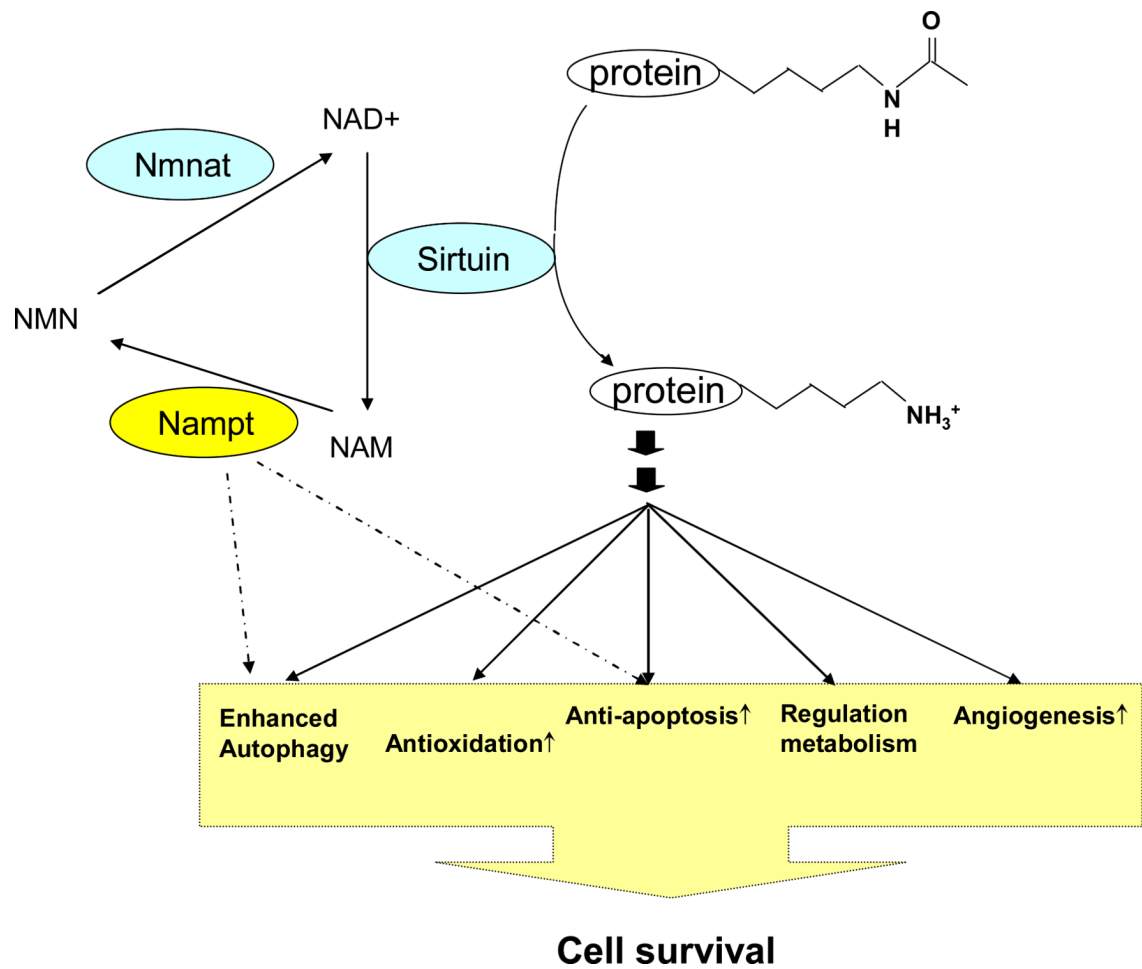
Figure 3.
The salvage pathway of NAD$^+$ biosynthesis facilitates cell survival through augmentation of sirtuin function. NAM, a natural inhibitor of sirtuin, is generated by sirtuin-mediated deacetylation of target proteins. Nampt converts from NAM to NMN, which is the rate limiting reaction in the salvage pathway of NAD$^+$ biosynthesis.
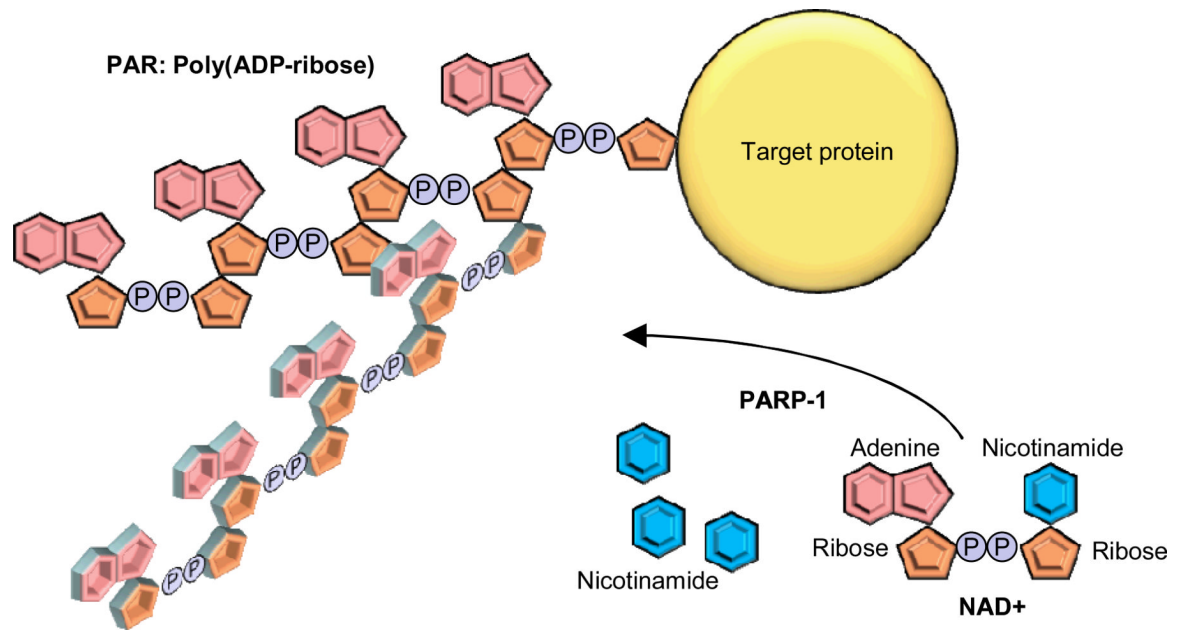
Figure 4.
Poly(ADP-ribosyl)ation (PAR) reaction. PARP-1 hydrolyses NAD$^+$ to generate ADP-ribose units and nicotinamides. The ADP-ribose units are polymerized and attached to target proteins. The generated PAR could be linear or branched.
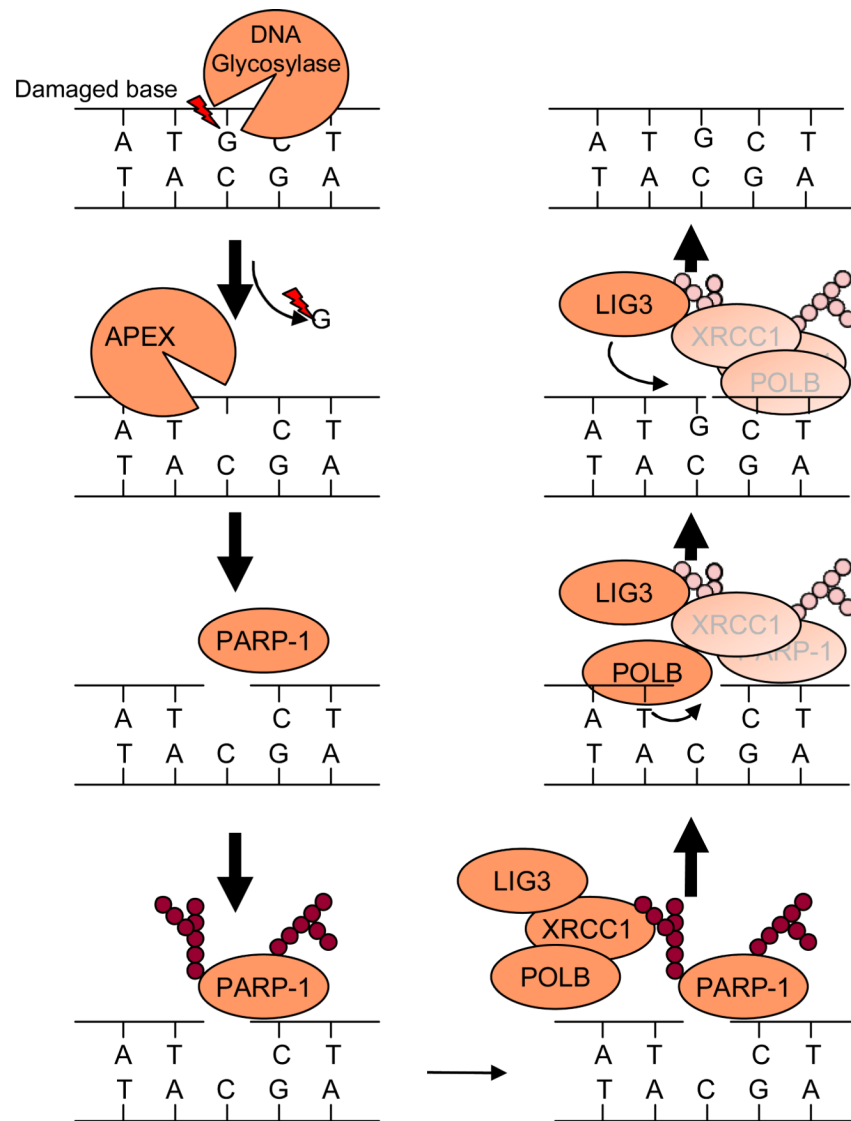
Figure 5.
The involvement of PARP-1 in base excision repair (BER). Damaged bases are recognized and cleaved by appropriate DNA glycosidase. The cleavage of an N-glycosidic bond by DNA glycosidase results in generation of apurinic or apyrmidinic site (AP site). DNA AP endonuclease or DNA AP lyase cleave the DNA backbone to create a single-strand DNA nick. PARP-1 recognizes the DNA nick as a single-strand break. The DNA binding activates PARP-1 to poly ADP-ribosylate target proteins, such as histone and PARP-1 itself. XRCC1, an important scaffold protein for BER, is recruited to where PARP-1 facilitates PAR since XRCC1 has a high affinity for PAR. XRCC1 binds to and recruits DNA polymerase beta (POLB) and DNA ligase III (LIG3) to the DNA nick. Then POLB fills the GAP and LIG3 ligases.
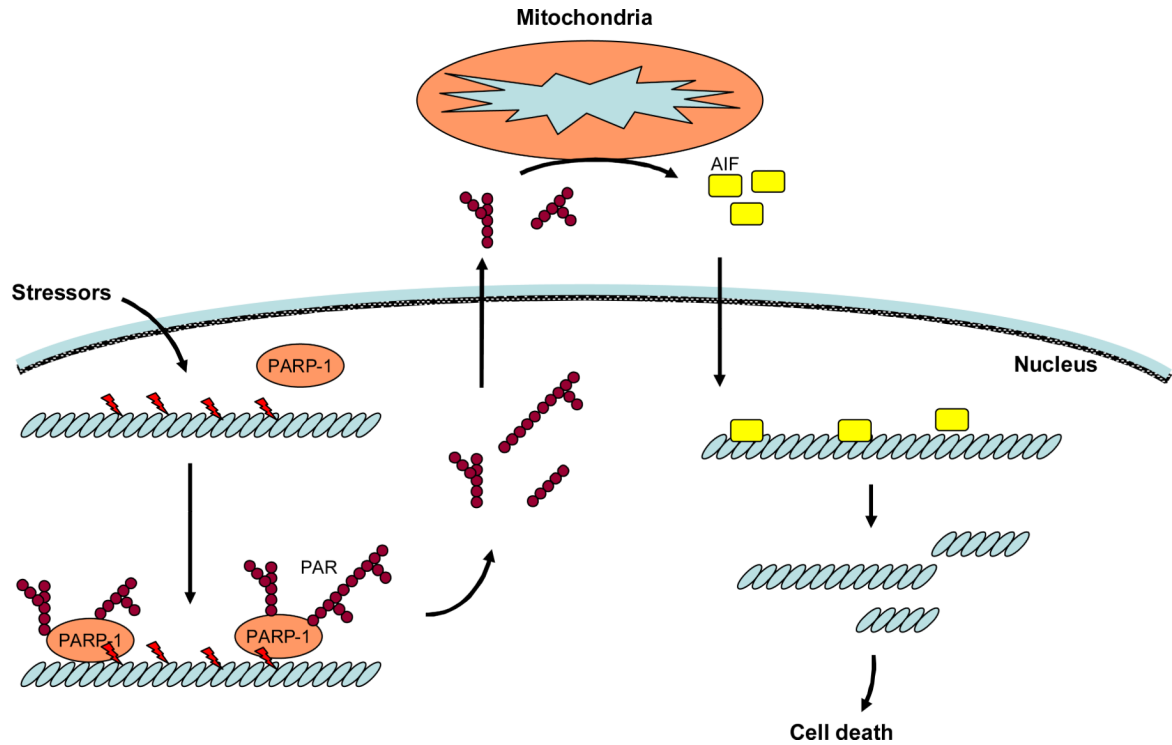
Figure 6.
PARP-1-induced caspase-independent cell death, termed parthanatos. Cell stressors, such as reactive oxygen species, cause DNA damage, which induces overactivation of PARP-1. PAR generated by PARP-1 activation translocates to the cytosol. Through direct interaction between PAR and mitochondria, apoptosis-inducing factor (AIF) is released from mitochondria and translocates to the nucleus. The AIF binds to DNA and induces large units of DNA fragmentation which is approximately 50 kbp.
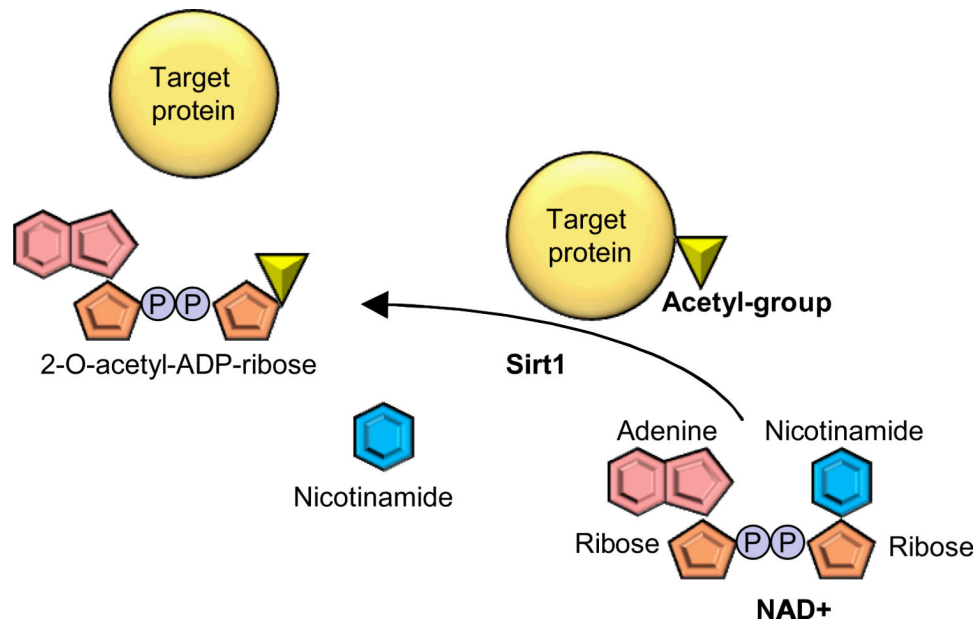
Figure 7.
Sirt1-mediated protein deacetylation. Sirt1 hydrolyses NAD+ to generate ADP-ribose units and nicotinamide. The acetyl group of the Sirt1 target proteins is removed and attached to the ADP-ribose to generate 2-O-acetyl-ADP-ribose.
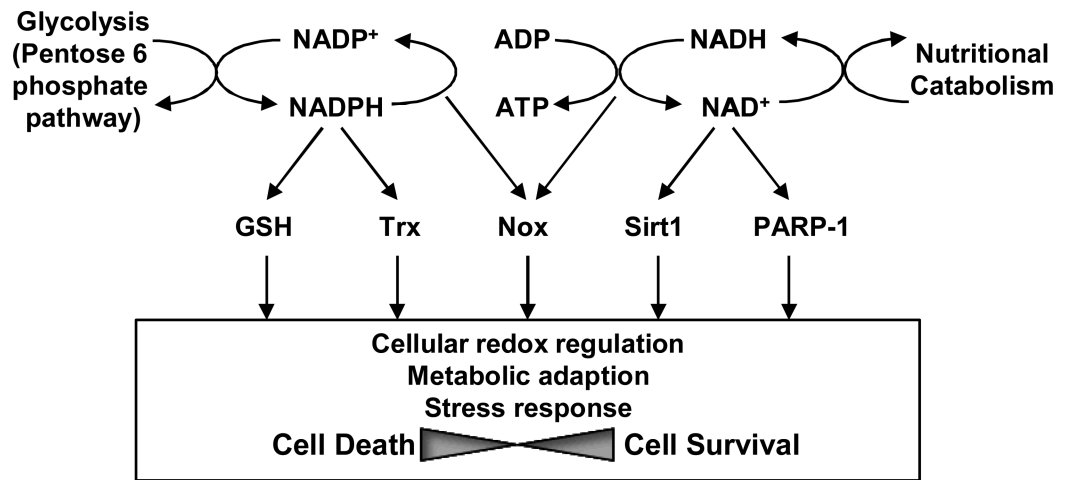
Figure 8.
Schematic representation of PNs. NADH and NADPH are high-energy intermediates for the transfer of energy from nutritional catabolism to cellular redox regulation and ATP production coupled with electron transfer. NADPH is mainly used for maintaining cellular redox status, whereas NADH is used for ATP production. NAD^+ is also used for providing ADP-ribose units for Sirt1 and PARP1, which is essential for the function of these enzymes. Although PNs have essential roles in cell survival, PARP-1 and Nox could be cell death inducers.