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Pyridine Nucleotide Homeostasis in Plant Development and Stress

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ABSTRACT

The traditional role of pyridine nucleotides (PNs) – NAD(H) and NADP(H), as the key redox couples in the cytoplasm of all living cells is well known. The reversible conversion between oxidized and reduced forms of PNs does not affect the net levels of these molecules. Resynthesis of these metabolites arises due to their participation in signaling reactions. Recent discoveries in animal systems have shown that derivatives of PNs such as ADP-ribose, cyclic ADP-ribose, *o*-acetyl ADP-ribose, nicotinic acid adenine dinucleotide phosphate are important signaling molecules. Some of these metabolites are reported in plant responses to environmental perturbations. In animal systems it has been shown that derivatives of PNs are involved in at least three important post-translational modifications of proteins – poly(ADP)-ribosylation, mono(ADP) ribosylation and *o*-acetylation. Role of these modifications in plants are gaining increasing attention. Levels of these PNs in plant cells show considerable changes during various developmental stages indicating these molecules can serve as metabolic read out of cell fate. Recent studies in animal systems show that the redox state of PNs can serve as global metabolic regulators of gene expression. Arabidopsis mutants with altered PN levels show massive changes in gene expression further supporting the animal studies. Our understanding of the role of PNs in plants is only beginning. A systems biology approach will facilitate a thorough understanding of the versatility of these labile redox molecules in plant health and sickness.

Keywords: ADP-ribose, metabolism, post-translational, redox, signaling
Abbreviations: **ADP**, adenine dinucleotide phosphate; **ADPR**, adenine dinucleotide phosphate ribose; **cADPR**, cyclic adenine dinucleotide phosphate ribose; **NAADP**, nicotinic acid adenine dinucleotide phosphate; ***o*-AADPR**, *o*-acetyl adenine dinucleotide phosphate ribose; **PARP**, poly(ADP) ribose polymerase; **PN**, pyridine nucleotide

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INTRODUCTION

Nicotinamide adenine dinucleotide (NAD) and its phosphorylated form, nicotinamide adenine dinucleotide phosphate (NADP), exist as oxidized form (NAD^+ or $NADP^+$, respectively) or in reduced form (NADH and NADPH, respectively) (**Fig. 1**). NAD^+, $NADP^+$, NADH and NADPH are together referred to as pyridine nucleotides (PNs). PNs are ubiquitous coenzymes involved in oxidation-reduction reactions in all organisms (Berger *et al.* 2004). They are central components of the cytoplasmic redox milieu and the reduction versus oxidation states of these metabolites serve as signals for evoking responses to internal developmental changes as well as external environmental perturbations (Dietz 2003).

The concentration of cytosolic NAD in plants is 0.6-0.7 mM, most of which exist in oxidized form, so that free NADH is barely detectable and estimated to be around 1 µM (Heineke *et al*. 1991; Wigge *et al*. 1993). On the contrary, the cytosolic NADP (0.2-0.3 mM) is mostly reduced, accounting for NADPH concentrations of 0.15-0.2 mM. The concentrations of NADP(H) is largely regulated by environmental conditions. The reduction state of chloroplast PNs is strongly influenced by light conditions in plants (Heber and Santarius 1965). In sugar beet (*Beta vulgaris*) and spinach (*Spinacea oleracea*), 40% of NADPH is in chloroplasts and increases to 60-85% under light conditions (Hunt *et al.* 2004). In another study with spinach (*S. oleracea*) leaves, chloroplast NADPH/$NADP^+$ was influenced by CO_2 concentrations supplied to the leaves (Dietz and Heber 1983). Chloroplast membrane is impermeable to exogenous PNs indicating that the pool of PNs in this organelle is adjusted internally, maybe by formation of phosphoglycerate during photosynthesis (Hunt *et al.* 2004). Interestingly, unlike in animals, the plant mitochondria are permeable to PNs (Douce and Neuburger 1989), indicating that cytosolic and mitochondrial NAD^+ concentrations are equilibrated. The nuclear membrane is freely permeable to PNs, suggesting that nuclear and cytoplasmic NADH:NAD^+ ratios are comparable (Fjeld *et al.* 2003). This can have a profound

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Invited Review

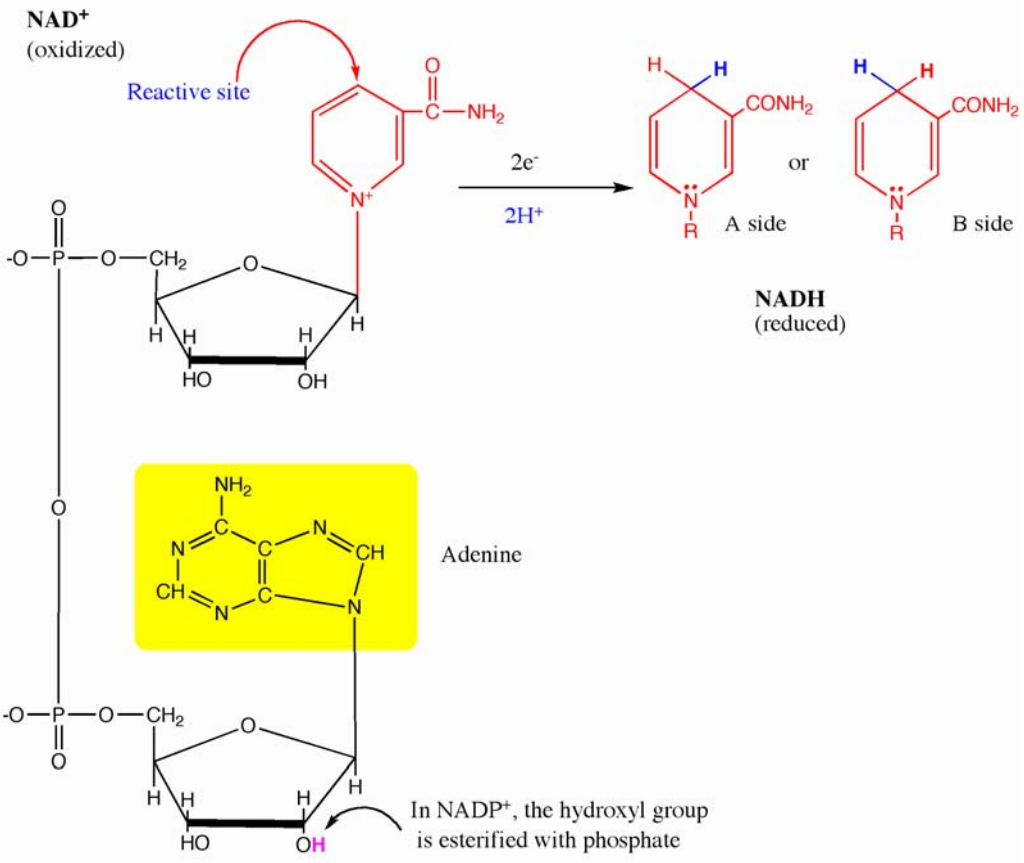

Fig. 1 Structure of pyridine nucleotides.

influence on gene regulation in response to alterations in cytosolic redox (discussed later).

Quantitation of PNs in photosynthetic cells is difficult because tissue NAD(P) concentrations are low and complex enzymatic cycling assays need to be used following the destruction of either oxidized or reduced form in alkali and acid, respectively (Lowry and Passonneau 1972; Matsumara and Miyachi 1980). Further, reliable determination of low concentrations of PNs depends on accelerated quenching of the metabolism due to rapid turnover of PNs in metabolic processes (Dietz 2003). An alternate method for estimating reduced NADP is based on the reaction equilibrium of the 3-phosphoglycerate (3-PGA) reduction, yielding dihydroxyacetone phosphate (DHAP) through the action of 3-PGA kinase, glyceraldehyde-3-phosphate dehydrogenase and triosephosphate isomerase, according to the equation below (Dietz and Heber 1989).

$$\frac{[3-PGA][NADPH][H^+][ATP]}{[DHAP][NADP^+][ADP][Pi]} = 5.4x10^{-6}$$

Activation of chloroplast malate dehydrogenase has been used to quantify the reduction state of NADP in this organelle (Holfgrefe *et al.* 1997). Sophisticated two-photon excitation microscopy has been used to quantitate source and number of subcellular NAD(P)H fluorescence in pancreatic islet cells by comparing with fluorescence from a purified NAD(P)H source (Patterson *et al*. 2000). Regulation of the C-terminal binding protein in mammalian cells by nuclear NADH levels was demonstrated using this technique (Zhang *et al*. 2002). Recently, a simple plate reader assay for the rapid analysis of PNs, as well as ascorbate and glutathione redox couples in plant extracts was reported (Queval and Noctor 2007). Development of such sensitive and rapid methods for measuring redox couples will be extremely useful for analyzing the changes in these labile metabolites during plant development and in response to various environmental perturbations.

SYNTHESIS OF PNs

Similar to purine and pyrimidine biosynthesis, PNs are synthesized by *de novo* and salvage pathways (Ashihara *et al*. 2005) (**Fig. 2**). In the *de novo* pathway, L-aspartic acid is oxidized to iminosuccinic acid by L-Asp oxidase enzyme (AO; EC 1.4.3.16). Condensation of iminosuccinate with glyceraldehyde-3-phosphate and cyclization to quinolinic acid is accomplished by quinolinate synthase (QS). Subsequently, phosphoribosyl pyrophosphate (PRPP) and quinolinic acid combine to produce nicotinic acid mononucleotide (NaMN) catalyzed by the enzyme quinolinic acid phosphoribosyl transferase (QPRT). These three enzymes, AO, QS and QPRT are encoded by single genes in the Arabidopsis genome, are localized in the chloroplast, and are indispensable for normal growth (Katoh *et al*. 2006). NaMN is converted to nicotinic acid adenine dinucelotide by nicotinamide mononucleotide adenylyl transferase (NaMNAT) and subsequently amidated to NAD by the action of NAD synthetase. The last two enzymes, NaMNAT and NAD synthetase, are encoded by single genes in Arabidopsis genome (Hunt *et al*. 2004).

In the salvage pathway, nicotinamide generated by the degradation of NAD, by the action of NAD glycohydrolases, NAD-dependent deacetylases, mono-ADP ribosyl transferases and polyADP ribose polymerases, is used for regenerating NAD. Nicotinamidases deamidate nicotinamide to nicotinic acid and transfers it to PRPP (Wang and Pichersky 2007). From this step, the enzymes involved in the NAD biosynthesis are the same as described above for the *de novo* pathway. Given the overlap between *de novo* pathway and salvage pathway, we predict that a loss-of-function of NaMNAT or NAD synthetase may be lethal. This needs to be tested using the T-DNA insertional mutants available for these two genes from the Arabidopsis database.

The molecular identification of nicotinamide riboside kinase in mammalian cells and yeast has established a novel route for NAD biosynthesis (Sasiak and Saunders 1996; Bieganowski and Brenner 2004). Presence of the nicotinamide riboside in food sources suggests that niacin or vita-

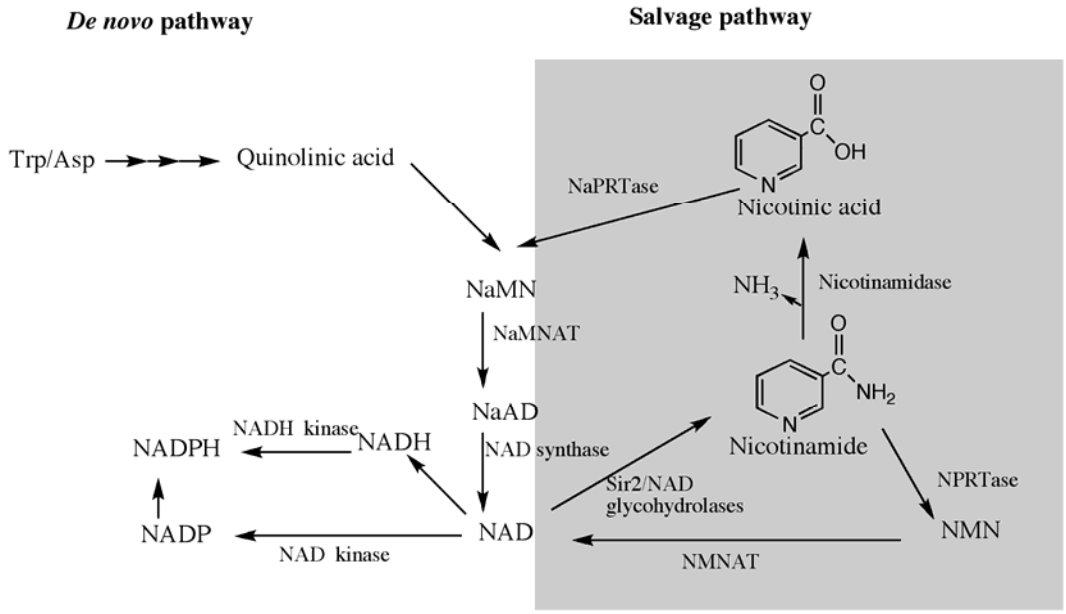

Fig. 2 Biosynthetic pathways of pyridine nucleotides. NaMN, nicotinic acid mono nucleotide; NaMNAT, nicotinic acid mono nucleotide adenylyl transferase; NaAD, nicotinic acid adenine dinucleotide; NPRTase, nicotinate-phosphoribosyltransferase.

min B3 may not be the only source for *de novo* synthesis of NAD in plants.

The only *de novo* mechanism for the formation of $NADP^+$ in plants is by NAD(H) kinases using NAD(H) and ATP as substrates (Delumeau *et al*. 2000; Gallais *et al*. 2001). There are three NAD(H) kinases in the Arabidopsis genome. AtNDK1 can utilize both NAD^+ and NADH as substrates and is induced in response to pathogens and oxidative stress (Berrin *et al*. 2005). AtNDK2 is described as NAD^+ kinase and it also binds calmodulin (Turner *et al*. 2004). AtNDK3 prefers NADH over NAD^+ and is inactivated by thiol-modifying reagents (Turner *et al*. 2005). The rapid changes in the $NAD^+/NADP^+$ in response to wounding and pathogens indicates that there must be specific $NADP^+$ phosphatases (Harding *et al*. 1997; Moller 2001). $NADP^+$ phosphatase which converts the $NADP^+$ to NAD^+ has been reported in the dormant seeds of *Avena sativa* (Gallais *et al*. 2001). Thus the kinase/phosphatase ratio can be an important determinant of the nicotinamide nucleotide metabolism (Turner *et al*. 2005).

PNs IN PLANT DEVELOPMENT

In higher plants the organs and tissues of different ages coexist in the same individual and PN levels serve as rate-limiting factors for regulating the metabolism in each part (Yamamoto 1963). In the storage organs such as endosperm and cotyledons, levels of NAD are very high and exist mostly in oxidized form, and NADP levels are very low, and most of it is in reduced form. In hypocotyls levels of NADP are strikingly high. The NADP+NADPH/ NAD+ NADH ratio is low in storage organs and high in growing parts of a plant. The NADPH/NADP ratio in cotyledon, hypocotyls, roots and leaves decreases with increasing age (Yamamoto 1963). In Arabidopsis plants, the NAD^+/NADH ratio showed a vast variation in different organs – roots, stem, rosette leaf, cauline leaf, flowers, silique), while the $NADP^+$/NADPH ratio remained fairly constant in these organs (Wang and Pichersky 2007).

Changes in the PN pool levels have been demonstrated to be important signals for rapidly switching the energy metabolism of plants from a vegetative to a reproductive stage (Bonzon *et al*. 1983). Using plate reader, four redox couples – NAD, NADP, ascorbate and glutathione were analyzed in Arabidopsis plants at five different time points spanning vegetative stage and four different time points during reproductive stage. NAD levels increased during the vegetative stage and plummeted during the flowering stage. However, the levels of NADH remained very stable (<20%) through out the course of this analysis. NADP levels were least variable both in terms of total contents and redox state (Queval and Noctor 2007). These studies indicate that the NAD and NADP redox couples show very little variation during Arabidopsis growth under controlled conditions. Nonetheless, the sampling frequency (nine time points over a period of 70-75 days) may be masking the subtle modulations in the pyridine nucleotide contents. Also if the sampling were done in the light versus the dark cycle, there may be significant differences in the levels of PNs (Ohhama and Miyachi 1959; Ogren and Krogmann 1965).

PNs IN PLANT RESPONSES TO STRESSES

NAD pools in plants are highly plastic and can undergo significant changes in response to the environment (Noctor *et al*. 2006). Changes in NAD levels can serve as important modulators of metabolic pathways especially during conditions of stress (Dutilleul *et al*. 2003a, 2003b, 2005) (**Fig. 3**). In barley leaves infected with the powdery mildew pathogen, *Erysiphae gramini* var. *hordei*, 2-fold increase in NAD levels were observed six days after inoculation and this increase was accompanied by an increase in the rate of respiration (Ryrie and Scott 1968). In this study it was reported that the content of $NADP^+$ in the non-chloroplast fraction was around 60% and this redistribution of the PN was responsible for the increase in activity of pentose phosphate pathway and respiration (Ryrie and Scott 1968). Using the French bean suspension-cultured cells treated with elicitors from fungal pathogen *Colletotrichum lindemuthianum*, a rapid increase in oxygen uptake was accompanied by an increase in hydrogen peroxide levels. This was followed by a rapid decline in the ATP levels and the NADH/NAD^+ ratios suggestive of a transient stress in oxidative metabolism and aerobic respiration (Robertson *et al*. 1995).

NADPH is important reducing energy equivalent required for several antioxidant defense systems. In Arabidopsis, cytosolic NADPH is generated by AtNADK3, a NADH kinase (Chai *et al*. 2006). Loss-of-function of *NADK3* renders the plants more sensitive to oxidative stress, further supporting the role of PNs especially NADPH for strengthening defense responses. Maintenance of high NADPH levels has been suggested to be an important factor in preventing injury in cold-hardened pea plants, hardened wheat plants subjected to drought stress and during cold acclimation in winter rape plants (Kuraishi *et al*. 1968; Maciejewska and Kacperska 1987; Zagdanska 1989). In transgenic rice plants over-expressing a NADPH-HC toxin reductase, synthesis of PNs were increased, which in turn led to enhanced tolerance to cell death by hydrogen peroxide the cytosolic Glycerol-3-phosphate dehydrogenase leads to

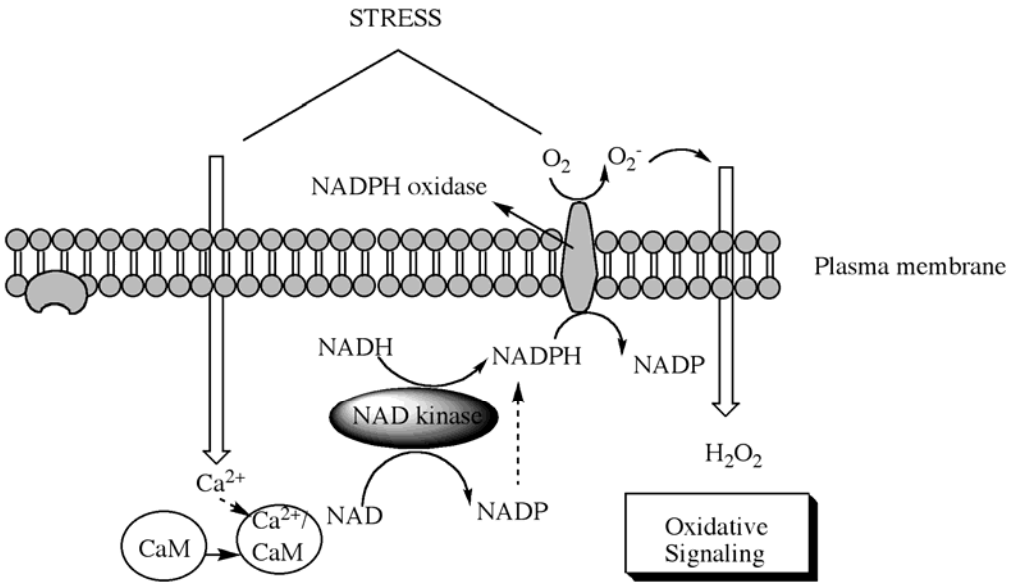

Fig. 3 Interconnections between reactive oxygen species, calcium and pyridine nucleotides. CaM, calmodulin, O^{2-}, superoxide; H_2O_2, hydrogen peroxide. Oxidative signaling includes changes in gene expression, enzyme activity, second messengers.

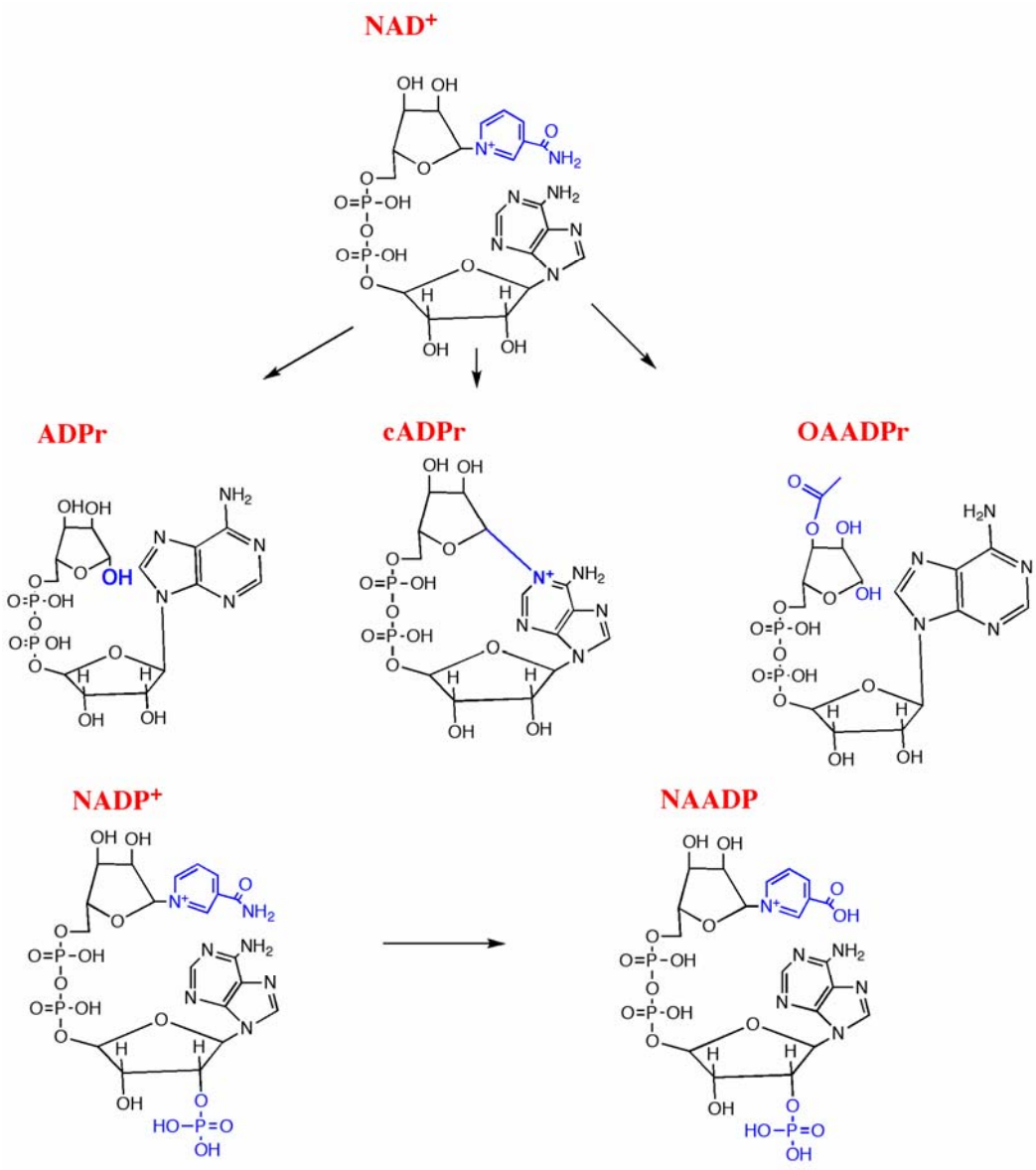

Fig. 4 Structures of pyridine nucleotide derived metabolites.

and bacterial pathogens (Hayashi *et al*. 2005). Mutations in increased NADH/NAD^+ ratios in the mutant plants leading to increased ROS levels and sensitivity to salt stress (Shen *et al*. 2006). The Arabidopsis nudix hydrolase *Atnudt7* hydrolyzes NADH and ADP-ribose *in-vitro* (Ogawa *et al*. 2005; Olejnik and Kraszewska 2005) Mutation in *Atnudt7* leads to increased ROS, NADH and increased resistance to virulent bacterial pathogens (Jambunathan and Mahalingam 2006). Poly(ADP-ribose) polymerase (PARP) enzyme induced by stress is a major consumer of NAD since this enzyme transfers ADP-ribose units from NAD to target proteins, releasing nicotinamide and forming long ADP-ribose chains with concomitant depletion of ATP (Amor *et al*. 1998; Graziani *et al*. 2005). RNAi lines with diminished PARP activity were shown to have higher energy-use efficiency by reducing mitochondrial respiration and ROS levels, that ultimately improved their tolerance to stresses (De Block *et al*. 2005). These studies clearly demonstrate that broad-spectrum tolerance to biotic and abiotic stresses can be engineered by manipulating PN biosynthesis and/or degradation pathways in plants.

PNs AND SIGNAL TRANSDUCTION

The reversible conversion of NAD(P) between oxidized and reduced forms does not impact the overall consumption of PNs in a cell. Hence re-synthesis of these metabolites does not arise due to its well-known role as redox carriers, but from their participation in cellular signaling reactions (Pollak *et al*. 2007). The increasing attention given to NAD in recent years stems from their ability to serve as precursors of several important second messenger molecules. This includes ADP-ribose (ADPR), cyclic ADP-ribose (cADPR), and *o*-acetyl ADP ribose (*o*-AADPR), all three of which are derived from NAD^+. Nicotinic acid adenine dinucleotide phosphate (NAADP), is the only NADP derived second messenger molecule (**Fig. 4**).

ADPR is a toxic metabolite that can lead to glycation and glyoxidation of long-lived intracellular proteins such as histones (Cervantes-Laurean *et al*. 1996). ADPR can be generated from NAD by several different metabolic pathways. The DNA breakage induced ADPR polymers synthesized by PARPS are rapidly turned over by polyADP-ribose glycohydrolases (PARGs), that can lead to ADPR accumulation. In the Arabidopsis genome, there are three genes that are annotated as PARPs and three genes as PARGs. Rapid induction of *parp1* and *parp2* during DNA breaks induced by ionizing radiation or oxidative stress has been reported (Doucet-Chabeaud *et al*. 2001). In soybean (*Glycine max*) cells expressing the antisense of *parp2* mRNA, cell death due to hydrogen peroxide was inhibited (Amor *et al*. 1998). However, direct evidence for changes in ADP-ribose metabolite levels in response to stresses in plants have not been reported yet. In animal systems the removal of ADPR moiety from proteins by protein mono-ADP-ribosyltransferases has been reported to be another source for ADPR. However, such mono-ADP-ribosyltransferase activity has not been demonstrated in plants yet.

cADPR plays a vital role in the modulation of calcium release from internal cellular stores (Lee and Aarhus 1991; Lee 1994; Lee *et al*. 1995). cADPR is synthesized from NAD by ADP-ribosyl cyclase enzyme (Lee 1994) while cyclic-ADP ribose hydrolases catalyze the breakdown of cyclic ADPR molecules leading to ADP ribose accumulation. The plant hormone ABA induces the activity of ADP-ribose cyclase within 15 minutes after treatment and nearly 28% of ABA-responsive genes are similarly regulated by cADPR (Sanchez *et al*. 2004). These studies established that cADPR is an important component of ABA signaling pathway (Wu *et al*. 1997; Sanchez *et al*. 2004). Further, the fact that more than 400 cADPR responsive genes act independent of the ABA pathway suggests that this signaling molecule may be playing an important role in other unknown signaling pathways.

o-AADPR is formed during the deacetylation reaction carried out by a group of proteins called as sirtuins that play a role in suppression of recombination, and ageing (Tanner *et al*. 2000; Denu 2003; Blander and Guarente 2004). *o*-AADPR has calcium mobilizing properties via activation of TRPM2 channel (Liou *et al*. 2005). In the Arabidopsis genome there are two potential homologues of sirtuins, however, these genes have not been characterized yet (Hunt *et al*. 2004) and no studies of *o*-AADPR in plants has been reported yet.

NAADP has been described as the most potent calcium-mobilizing messenger (Yamasaki *et al*. 2005). NAADP mediated calcium release has been shown in the microsomal vesicles of red beets (*Beta vulgaris*) and cauliflower (*Brassica oleracea*) (Navazio *et al*. 2000). This nonvacuolar pathway for calcium relase distinct from the inositol phosphates and cADPR gated pathways, provides an other important facet of the complex calcium signatures mediated by these distinct second messengers. *In vitro*, NAADP synthesis required high levels of nicotinic acid (30 mM) and low pH (4-5) (Lee 2000), both of which are not physiologically relevant. This suggests that alternative routes for their biosynthesis may exist in plants and animals and needs to be determined.

PNs AND PROTEIN MODIFICATIONS

Three important protein modifications are brought about by the second messenger molecules derived from PNs – poly (ADP-ribosyl)ation, mono(ADP-ribosyl)ation and protein deacetylation (**Fig. 5**).

Poly(ADP-ribosyl)ation is catalyzed by PARPs (Jacobson and Jacobson 1999; Burkle 2001). Using NAD as substrates, these enzymes form branched polymers of ADP-ribose that are then attached to glutamate residues of acceptor proteins. Based on studies in animal systems, PARPs are involved in a gamut of cellular processes such as DNA repair, cell cycle regulation, apoptosis and maintenance of chromosome length (Burkle 2001; Virag and Szabo 2002; Chiarugi and Moskowitz 2003). Based on studies in various plant species, PARPs are important for induction of defense mechanisms (Berglund *et al*. 1996) and in response to different stresses (Amor *et al*. 1998; Doucet-Chabeaud *et al*. 2001). Using PARP inhibitors and antisense approach it was shown that Brassica plants with reduced PARP activity have higher energy levels and are more resistant to abiotic stresses like high light, drought and heat (De Block *et al*. 2005). Though these studies demonstrated a role for PARPs in stress signaling and DNA repair, the targets of poly ADP-ribosylation are not known.

Mono(ADP) ribosylation refers to the enzymatic transfer of ADPR from NAD^+ to acceptor proteins (Corda and Di Girolamo 2003). These are catalyzed by cellular ADP-ribosyltransferases. ADP-ribosylation usually leads to protein inactivation, providing a mechanism for inhibiting protein functions in both physiological and stress conditions. The identification of ADP-ribosylhydrolases that reverse the reaction by hydrolyzing the protein-ADP-ribose linkage suggests that reversible protein mono-ADP-ribosylation acts as a regulatory mechanism for such proteins e.g. G-protein beta subunit, actin, tubulin and desmin (Corda and Di Girolamo 2003; Di Girolamo *et al*. 2005). To date, not a single plant ADP-ribosyltransferase protein has been functionally characterized. Based on their domain organization, Arabidopsis 'radical induced cell death' protein (*RCD1*) has been suggested to function as a mono-ADP-ribosyltransferase (Ahlfors *et al*. 2004), however the biochemical evidence for this activity has not been demonstrated yet.

Protein deacetylation is carried out by sirtuin proteins that catalyze the NAD-nicotinamide exchange reaction that requires the acetylated lysine found in the N-terminus of histones. For every acetyl group removed from a lysine molecule, one molecule of NAD is hydrolyzed to form a molecule of nicotinamide (Tanny and Moazed 2001). As described earlier the acetyl group is transferred to ADP-ribose to give rise to a new signaling molecule called *O*-

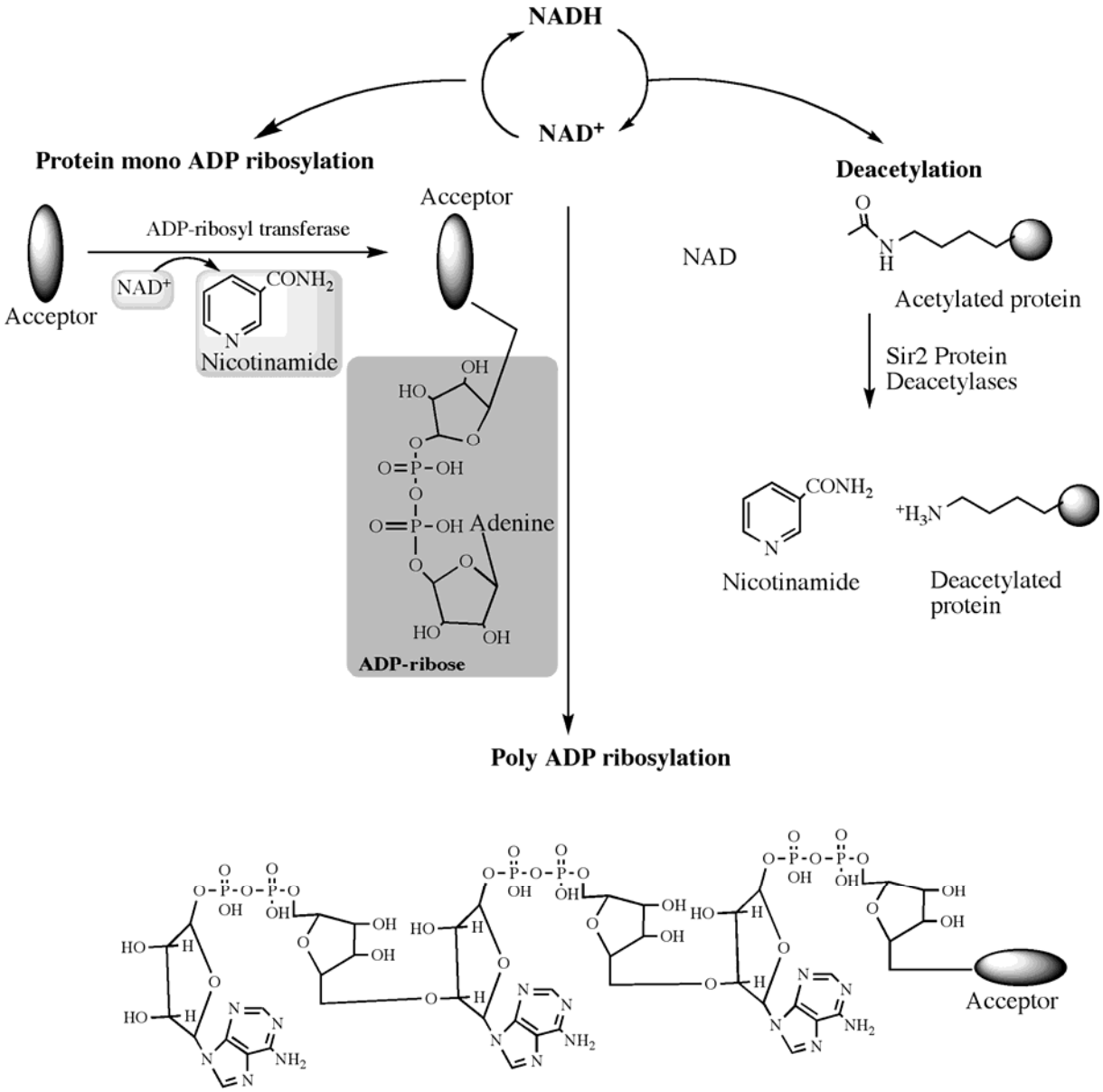

Fig. 5 Common post-translational modification reactions involving pyridine nucleotides.

AADPR. There are two potential Arabidopsis homologues of Sir2 and both lack nuclear localization signals suggesting its substrate may be non-histone cytosolic or mitochondrial proteins (Hunt *et al*. 2004). Yeast Sir2 is regulated by plant polyphenols (Howitz *et al*. 2003). Since plants produce a variety of polyphenols, especially in response to stresses, it has been speculated that these compounds can act as sir2 regulators in plants (Hunt *et al*. 2004).

PNs AND GENE REGULATION

In animal systems, NAD salvage pathway plays an important role in transcriptional silencing at the telomere and rDNA loci (Smith and Boeke 1997; Anderson *et al*. 2002; Sandmeier *et al*. 2002). Sir2p activity is required for this silencing, which requires NAD (Lin and Guarente 2003). Since salvage pathway proteins are localized to the nucleus it has been suggested that NAD can be readily utilized by Sir2p. Functional characterization of the Sir2 proteins in plants will aid in determining if such global transcriptional silencing at the telomeres and rDNA loci can be regulated by PNs.

NAD redox status plays an important role in regulating the transcription factors involved in circadian control in animals (Rutter *et al*. 2001). In Arabidopsis, mutation at the *tej* loci affects clock-controlled genes and alters the timing of photoperiod dependent transition from vegetative to flowering stage (Panda *et al*. 2002). TEJ encodes a poly (ADP-ribose) glycohydrolase protein suggesting that post-translational poly(ADP-ribosyl)ation of an oscillator component contribute to setting the period of a central oscillator in Arabidopsis (Panda *et al*. 2002). As indicated earlier identification of the target proteins for poly (ADP-ribosyl) ation will enable a clear understanding of the role of PNs, in the establishment of period length in plants.

The carboxyl-terminal binding protein (CtBP) a strong co-repressor that binds to transcriptional repressors is regulated by PNs, especially the levels of nuclear NADH (Zhang *et al*. 2002). Mutation in two different Arabidopsis genes – cytosolic glyceraldhyde–3-phospate dehydrogenase and nudix hydrolase 7 that alter the NADH levels in the plants have been described recently (Jambunathan and Mahalingam 2006; Shen *et al*. 2006). A T-DNA insertion line in the promoter region of the Arabidopsis nudix hydrolase 7 (*NUDT7*) gene does not cause any phenotypic abnormalities in the mutant plant (Jambunathan and Mahalingam 2006). NUDT7 is a NADH and ADP-ribose pyrophosphatase (Ogawa *et al*. 2005; Jambunathan and Mahalingam 2006). In the *nudt7* knock-down lines the levels of NADH were higher than in the wild-type plants (Jambunathan and Ma-

Fig. 6 Ratio-intensity plot. Log_2 of the ratio between the *nudt7* knock down mutant and wild-type gene expression values is plotted against the product of log_{10} intensity values of mutant and wildtype. The hybridizations were performed using Arabidopsis Affymetrix ATH1 gene chips using two chips for each sample.

halingam, unpublished data). Whole-genome microarray analysis of the *nudt7* knock-down plants in comparison with wild-type plants revealed 670 genes were more than 2-fold repressed in the mutant (**Fig. 6**). Importantly, the gene ontology category of transcriptional regulation was significantly enriched among the genes repressed in the knock-down plants, further implicating a role for PNs in global gene regulation in plants. Microarray analysis using other mutants with altered levels of PNs should enable cogent identification of transcriptionally regulated cellular targets that are directly controlled by PNs in plants using a 'guilt-by-association' approach.

CONCLUDING REMARKS

PNs are the most versatile redox molecules essential for energy transduction in a cell. Studies in animal system and yeast have led to the identification of PN derivative molecules that play vital regulatory roles, indispensable for normal growth of a cell and in responses to perturbations in the environment. The signaling role of PNs in plant development and stress signaling is gaining increasing attention. The finding that the PN levels are highly plastic in a plant cell and potentially can act as a read out of cell fate underscores the importance of these molecules. Our current understanding of the role of PNs in a cell can only be described as the tip of the ice-berg. A combination of forward and reverse genetic approaches in conjunction with metabolite profiling is necessary to dissect the complex cellular redox network coordinated by PNs and its derivatives.

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REFERENCES

Ahlfors R, Lang S, Overmyer K, Jaspers P, Brosche M, Taurianinen A, Kollist H, Tuominen H, Belles-Boix E, Piippo M, Inze M, Palva ET, Kangasjarvi J (2004) Arabidopsis *radical-induced cell death1* belongs to the WWE protein-protein interaction domain protein family and modulates abscisic acid, ethylene, and methyl jasmonate responses. *Plant Cell* **16**, 1925-1937

Amor Y, Babiychuk E, Inzé D, Levine A (1998) The involvement of poly (ADP-ribose) polymerase in the oxidative stress responses in plants. *FEBS Letters* **440**, 1-7

Anderson RM, Bitterman KJ, Wood JG, Medvedik O, Cohen H, Lin SS, Manchester JK, Gordon JI, Sinclair DA (2002) Manipulation of a nuclear NAD(+) salvage pathway delays aging without altering steady-state NAD(+) levels. *The Journal of Biological Chemistry* **277**, 18881-18890

Ashihara H, Stasolla C, Yin YL, Loukanina N, Thorpe TA (2005) *De novo* and salvage biosynthetic pathways of pyridine nucleotides and nicotinic acid conjugates in cultured plant cells. *Plant Science* **169**, 107-114

Berger F, Ramirez-Hernandez MH, Ziegler M (2004) The new life of a centenarian: signalling functions of NAD(P). *Trends in Biochemical Sciences* **29**, 111-118

Berglund T, Kalbin G, Strid A, Rydstrom J, Ohlsson AB (1996) UV-B- and oxidative stress-induced increase in nicotinamide and trigonelline and inhibition of defensive metabolism induction by poly(ADP-ribose)polymerase inhibitor in plant tissue. *FEBS Letters* **380**, 188-193

Berrin JG, Pierrugues O, Brutesco C, Alonso B, Montillet JL, Roby D, Kazmaier M (2005) Stress induces the expression of *AtNADK-1*, a gene encoding a NAD(H) kinase in *Arabidopsis thaliana*. *Molecular Genetics and Genomics* **273**, 10-19

Bieganowski P, Brenner C (2004) Discoveries of nicotinamide riboside as a nutrient and conserved NRK genes establish a Preiss-Handler independent route to NAD(+) in fungi and humans. *Cell* **117**, 495-502

Blander G, Guarente L (2004) The Sir2 family of protein deacetylases. *Annual Review of Biochemistry* **73**, 417-435

Bonzon M, Simon P, Greppin H, Wagner E (1983) Pyridine-nucleotides and redox charge evolution during the induction of flowering in Spinach leaves. *Planta* **159**, 254-260

Burkle A (2001) PARP-1: a regulator of genomic stability linked with mammalian longevity. *Chemical Biochemistry* **2**, 725-728

Cervantes-Laurean D, Jacobson EL, Jacobson MK (1996) Glycation and glycoxidation of histones by ADP-ribose. *The Journal of Biological Chemistry* **271**, 10461-10469

Chai MF, Wei PC, Chen QJ, An R, Chen J, Yang S, Wang XC (2006) *NADK3*, a novel cytoplasmic source of NADPH, is required under conditions of oxidative stress and modulates abscisic acid responses in Arabidopsis. *The Plant Journal* **47**, 665-674

Chiarugi A, Moskowitz MA (2003) Poly(ADP-ribose) polymerase-1 activity promotes NF-kappaB-driven transcription and microglial activation: implication for neurodegenerative disorders. *Journal of Neurochemistry* **85**, 306-317

Corda D, Di Girolamo M (2003) Functional aspects of protein mono-ADP-ribosylation. *The EMBO Journal* **22**, 1953-1958

De Block M, Verduyn C, De Brouwer D, Cornelissen M (2005) Poly(ADP-ribose) polymerase in plants affects energy homeostasis, cell death and stress tolerance. *The Plant Journal* **41**, 95-106

Delumeau O, Renard M, Montrichard F (2000) Characterization and possible redox regulation of the purified calmodulin-dependent NAD(+) kinase from *Lycopersicon pimpinellifolium*. *Plant Cell and Environment* **23**, 1267-1273

Denu JM (2003) Linking chromatin function with metabolic networks: Sir2 family of NAD(+)-dependent deacetylases. *Trends in Biochemical Sciences* **28**, 41-48

Di Girolamo M, Dani N, Stilla A, Corda D (2005) Physiological relevance of the endogenous mono(ADP-ribosyl)ation of cellular proteins. *FEBS Journal* **272**, 4565-4575

Dietz KJ (2003) Redox control, redox signaling and redox homeostasis in plant cells. *International Review of Cytology* **228**, 141-193

Dietz KJ, Heber U (1983) Rate-limiting factors in leaf photosynthesis. I. Carbon fluxes in the calvin cycle. *Biochimica et Biphysica Acta* **767**, 432-443

Dietz KJ, Heber U (1989) Assimilatory force and regulation of photosynthetic carbon reduction in plants. In: Barber J (Ed) *Techniques and New Developments in Photosynthesis*, NATO ASI Series New York, pp 341-363

Douce R, Neuburger M (1989) The uniqueness of plant-mitochondria. *Annual Review of Plant Physiology and Plant Molecular Biology* **40**, 371-414

Doucet-Chabeaud G, Godon C, Brutesco C, de Murcia G, Kazmaier M (2001) Ionising radiation induces the expression of *PARP-1* and *PARP-2* genes in Arabidopsis. *Molecular Genetics and Genomics* **265**, 954-963

Dutilleul C, Driscoll S, Cornic G, De Paepe R, Foyer CH, Noctor G (2003a) Functional mitochondrial complex I is required by tobacco leaves for optimal photosynthetic performance in photorespiratory conditions and during transients. *Plant Physiology* **131**, 264-275

Dutilleul C, Lelarge C, Prioul JL, De Paepe R, Foyer CH, Noctor G (2005) Mitochondria-driven changes in leaf NAD status exert a crucial influence on the control of nitrate assimilation and the integration of carbon and nitrogen metabolism. *Plant Physiology* **139**, 64-78

Dutilleul C, Garmier M, Noctor G, Mathieu C, Chetrit P, Foyer CH, de Paepe R (2003b) Leaf mitochondria modulate whole cell redox homeostasis, set antioxidant capacity, and determine stress resistance through altered signaling and diurnal regulation. *Plant Cell* **15**, 1212-1226

Fjeld CC, Birdsong WT, Goodman RH (2003) Differential binding of NAD (+) and NADH allows the transcriptional corepressor carboxyl-terminal binding protein to serve as a metabolic sensor. *Proceedings of the National Academy of Sciences USA* **100**, 9202-9207

Gallais S, de Crescenzo MAP, Laval-Martin DL (2001) Characterization of soluble calcium calmodulin-dependent and -independent NAD(+) kinases from *Avena sativa* seeds. *Australian Journal of Plant Physiology* **28**, 363-371

Graziani G, Battaini F, Zhang J (2005) PARP-1 inhibition to treat cancer, ischemia, inflammation. *Pharmacological Research* **52**, 1-4

Harding SA, Oh SH, Roberts DM (1997) Transgenic tobacco expressing a foreign calmodulin gene shows an enhanced production of active oxygen species. *The EMBO Journal* **16**, 1137-1144

Hayashi M, Takahashi H, Tamura K, Huang JR, Yu LH, Kawai-Yamada M, Tezuka T, Uchimiya H (2005) Enhanced dihydroflavonol-4-reductase activity and NAD homeostasis leading to cell death tolerance in transgenic rice. *Proceedings of the National Academy of Sciences USA* **102**, 7020-7025

Heber U, Santarius KA (1965) Compartmentation and reduction of pyridine nucleotides in relation to photosynthesis. *Biochimica et Biphysica Acta* **109**, 390-408

Heineke D, Riens B, Grosse H, Hoferichter P, Peter U, Flugge UI, Heldt HW (1991) Redox transfer across the inner chloroplast envelope membrane. *Plant Physiology* **95**, 1131-1137

Holfgrefe S, Backhausen JE, Kitzmann C, Scheibe R (1997) Regulation of steady-state photosynthesis in isolated chloroplasts under constant light: Responses of carbon fluxes, metabolite pools and enzyme activation states to changes of electron pressure. *Plant and Cell Physiology* **38**, 1207-1216

Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG, Zipkin RE, Chung P, Kisielewski A, Zhang LL, Scherer B, Sinclair DA (2003) Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature* **425**, 191-196

Hunt L, Lerner F, Ziegler M (2004) NAD – new roles in signalling and gene regulation in plants. *New Phytologist* **163**, 31-44

Jacobson MK, Jacobson EL (1999) Discovering new ADP-ribose polymer cycles: protecting the genome and more. *Trends in Biochemical Sciences* **24**, 415-417

Jambunathan N, Mahalingam R (2006) Analysis of Arabidopsis *Growth Factor Gene 1* (*GFG1*) encoding a nudix hydrolase during oxidative signaling. *Planta* **224**, 1-11

Katoh A, Uenohara K, Akita M, Hashimoto T (2006) Early steps in the biosynthesis of NAD in Arabidopsis start with aspartate and occur in the plastid. *Plant Physiology* **141**, 851-857

Kuraishi S, Arai N, Ushijima T, Tazaki T (1968) Oxidized and reduced nicotinamide adenine dinucleotide phosphate levels of plants hardened and unhardened against chilling injury. *Plant Physiology* **43**, 238-242

Lee HC (1994) Cyclic ADP-Ribose – a calcium mobilizing metabolite of NAD(+). *Molecular and Cellular Biochemistry* **138**, 229-235

Lee HC (2000) NAADP: An emerging calcium signaling molecule. *Journal of Membrane Biology* **173**, 1-8

Lee HC, Aarhus R (1991) ADP-Ribosyl Cyclase – an enzyme that cyclizes NAD^+ into a calcium-mobilizing metabolite. *Cell Regulation* **2**, 203-209

Lee HC, Graeff R, Walseth TF (1995) Cyclic ADP-Ribose and its metabolic enzymes. *Biochimie* **77**, 345-355

Lin SJ, Guarente L (2003) Nicotinamide adenine dinucleotide, a metabolic regulator of transcription, longevity and disease. *Current Opinion in Cell Biology* **15**, 241-246

Liou JC, Ho SY, Shen MR, Liao YP, Chiu WT, Kang KH (2005) A rapid, nongenomic pathway facilitates the synaptic transmission induced by retinoic acid at the developing synapse. *Journal of Cell Science* **118**, 4721-4730

Lowry OH, Passonneau JV (1972) *A Flexible System of Enzymatic Analysis,* Academic Press, NY

Maciejewska U, Kacperska A (1987) Changes in the level of oxidized and reduced pyridine-nucleotides during cold-acclimation of winter rape plants. *Physiologia Plantarum* **69**, 687-691

Matsumara H, Miyachi S (1980) Cycling assay for nicotinamide adenine dinucleotides. *Methods in Enzymology* **69**, 465-470

Moller IM (2001) Plant mitochondria and oxidative stress: Electron transport, NADPH turnover, and metabolism of reactive oxygen species. *Annual Review of Plant Physiology and Plant Molecular Biology* **52**, 561-591

Navazio L, Bewell MA, Siddiqua A, Dickinson GD, Galione A, Sanders D (2000) Calcium release from the endoplasmic reticulum of higher plants elicited by the NADP metabolite nicotinic acid adenine dinucleotide phosphate. *Proceedings of the National Academy of Sciences USA* **97**, 8693-8698

Noctor G, Queval G, Gakiere B (2006) NAD(P) synthesis and pyridine nucleotide cycling in plants and their potential importance in stress conditions. *Journal of Experimental Botany* **57**, 1603-1620

Ogawa T, Ueda Y, Yoshimura K, Shigeoka S (2005) Comprehensive analysis of cytosolic nudix hydrolases in *Arabidopsis thaliana*. *The Journal of Biological Chemistry* **280**, 25277-25283

Ogren WL, Krogmann DW (1965) Studies on pyridine nucleotides in photosynthetic tissue. Concentrations, interconversion and distribution *The Journal of Biological Chemistry* **240**, 4603-4608

Ohhama T, Miyachi S (1959) Effects of illumination and oxygen supply upon the levels of pyridine nucleotides in Chlorella cells. *Biochimica et Biophysica Acta* **34**, 202-210

Olejnik K, Kraszewska E (2005) Cloning and characterization of an *Arabidopsis thaliana* nudix hydrolase homologous to the mammalian GFG protein. *Biochim Biophys Acta* **1752**, 133-141

Panda S, Poirier GG, Kay SA (2002) *tej* defines a role for poly(ADP-ribosyl) ation in establishing period length of the Arabidopsis circadian oscillator. *Developmental Cell* **3**, 51-61

Patterson GH, Knobel SM, Arkhammar P, Thastrup O, Piston DW (2000) Separation of the glucose-stimulated cytoplasmic and mitochondrial NAD(P) H responses in pancreatic islet beta cells. *Proceedings of the National Academy of Sciences USA* **97**, 5203-5207

Pollak N, Dolle C, Ziegler M (2007) The power to reduce: pyridine nucleotides-small molecules with a multitude of functions. *Biochemical Journal* **402**, 205-218

Queval G, Noctor G (2007) A plate reader method for the measurement of NAD, NADP, glutathione, and ascorbate in tissue extracts: Application to redox profiling during Arabidopsis rosette development. *Analytical Biochemistry* **363**, 58-69

Robertson D, Davies DR, Gerrish C, Jupe SC, Bolwell GP (1995) Rapid changes in oxidative metabolism as a consequence of elicitor treatment of suspension- cultured cells of French bean (*Phaseolus vulgaris* L.). *Plant Molecular Biology* **27**, 59-67

Rutter J, Reick M, Wu LC, McKnight SL (2001) Regulation of clock and NPAS2 DNA binding by the redox state of NAD cofactors. *Science* **293**, 510-514

Ryrie IJ, Scott KJ (1968) Metabolic regulation in diseased leaves. 2. Changes in nicotinamide nucleotide coenzymes in barley leaves infected with powdery mildew. *Plant Physiology* **43**, 687-692

Sanchez JP, Duque P, Chua NH (2004) ABA activates ADPR cyclase and cADPR induces a subset of ABA-responsive genes in Arabidopsis. *Plant Journal* **38**, 381-395

Sandmeier JJ, Celic I, Boeke JD, Smith JS (2002) Telomeric and rDNA silencing in *Saccharomyces cerevisiae* are dependent on a nuclear NAD(+) salvage pathway. *Genetics* **160**, 877-889

Sasiak K, Saunders PP (1996) Purification and properties of a human nicotinamide ribonucleoside kinase. *Archives of Biochemistry and Biophysics* **333**, 414-418

Shen W, Wei Y, Dauk M, Tan Y, Taylor DC, Selvaraj G, Zou J (2006) Involvement of a glycerol-3-phosphate dehydrogenase in modulating the NADH/NAD+ ratio provides evidence of a mitochondrial glycerol-3-phosphate shuttle in Arabidopsis. *Plant Cell* **18**, 422-441

Smith JS, Boeke JD (1997) An unusual form of transcriptional silencing in yeast ribosomal DNA. *Genes and Development* **11**, 241-254

Tanner KG, Landry J, Sternglanz R, Denu JM (2000) Silent information regulator 2 family of NAD- dependent histone/protein deacetylases generates a unique product, 1-O-acetyl-ADP-ribose. *Proceedings of the National Academy of Sciences USA* **97**, 14178-14182

Tanny JC, Moazed D (2001) Coupling of histone deacetylation to NAD breakdown by the yeast silencing protein Sir2: Evidence for acetyl transfer from substrate to an NAD breakdown product. *Proceedings of the National Academy of Sciences USA* **98**, 415-420

Turner WL, Waller JC, Snedden WA (2005) Identification, molecular cloning and functional characterization of a novel NADH kinase from *Arabidopsis thaliana* (thale cress). *Biochemical Journal* **385**, 217-223

Turner WL, Waller JC, Vanderbeld B, Snedden WA (2004) Cloning and characterization of two NAD kinases from arabidopsis. Identification of a calmodulin binding isoform. *Plant Physiology* **135**, 1243-1255

Virag L, Szabo C (2002) The therapeutic potential of poly(ADP-ribose) polymerase inhibitors. *Pharmacology Review* **54**, 375-429

Wang GD, Pichersky E (2007) Nicotinamidase participates in the salvage pathway of NAD biosynthesis in Arabidopsis. *The Plant Journal* **49**, 1020-1029

Wigge B, Kromer S, Gardestrom P (1993) The redox levels and subcellular-distribution of pyridine-nucleotides in illuminated barley leaf protoplasts studied by rapid fractionation. *Physiologia Plantarum* **88**, 10-18

Wu Y, Kuzma J, Marechal E, Graeff R, Lee HC, Foster R, Chua NH (1997) Abscisic acid signaling through cyclic ADP-Ribose in plants. *Science* **278**, 2126-2130

Yamamoto Y (1963) Pyridine nucleotide content in higher plant. Effect of age of tissue. *Plant Physiology* **38**, 45-54

Yamasaki M, Thomas JM, Churchill GC, Garnham C, Lewis AM, Cancela JM, Patel S, Galione A (2005) Role of NAADP and cADPR in the induction and maintenance of agonist-evoked Ca^{2+} spiking in mouse pancreatic acinar cells. *Current Biology* **15**, 874-878

Zagdanska B (1989) Effect of water-stress upon the pyridine-nucleotide pool in wheat leaves. *Journal of Plant Physiology* **134**, 320-326

Zhang Q, Piston DW, Goodman RH (2002) Regulation of corepressor function by nuclear NADH. *Science* **295**, 1895-1897