Title

Regulation of NAD+ metabolism, signaling and compartmentalization in the yeast Saccharomyces cerevisiae

Authors

Kato, Michiko
Lin, Su-Ju

Publication Date

2014-11-01

DOI

10.1016/j.dnarep.2014.07.009

Peer reviewed

Published in final edited form as:
DNA Repair (Amst). 2014 November ; 0: 49–58. doi:10.1016/j.dnarep.2014.07.009.

Regulation of NAD+ metabolism, signaling and compartmentalization in the yeast *Saccharomyces cerevisiae*

Michiko Kato and **Su-Ju Lin***
Department of Microbiology and Molecular Genetics, College of Biological Sciences, University of California, One Shields Ave. Davis, CA 95616, USA

Abstract

Pyridine nucleotides are essential coenzymes in many cellular redox reactions in all living systems. In addition to functioning as a redox carrier, NAD^+ is also a required co-substrate for the conserved sirtuin deacetylases. Sirtuins regulate transcription, genome maintenance and metabolism and function as molecular links between cells and their environment. Maintaining NAD^+ homeostasis is essential for proper cellular function and aberrant NAD^+ metabolism has been implicated in a number of metabolic- and age-associated diseases. Recently, NAD^+ metabolism has been linked to the phosphate-responsive signaling pathway (*PHO* pathway) in the budding yeast *Saccharomyces cerevisiae*. Activation of the *PHO* pathway is associated with the production and mobilization of the NAD^+ metabolite nicotinamide riboside (NR), which is mediated in part by *PHO*-regulated nucleotidases. Cross-regulation between NAD^+ metabolism and the *PHO* pathway has also been reported; however, detailed mechanisms remain to be elucidated. The *PHO* pathway also appears to modulate the activities of common downstream effectors of multiple nutrient-sensing pathways (Ras-PKA, TOR, Sch9/AKT). These signaling pathways were suggested to play a role in calorie restriction-mediated beneficial effects, which have also been linked to Sir2 function and NAD^+ metabolism. Here, we discuss the interactions of these pathways and their potential roles in regulating NAD^+ metabolism. In eukaryotic cells, intracellular compartmentalization facilitates the regulation of enzymatic functions and also concentrates or sequesters specific metabolites. Various NAD^+-mediated cellular functions such as mitochondrial oxidative phosphorylation are compartmentalized. Therefore, we also discuss several key players functioning in mitochondrial, cytosolic and vacuolar compartmentalization of NAD^+ intermediates, and their potential roles in NAD^+ homeostasis. To date, it remains unclear how NAD^+ and NAD^+ intermediates shuttle between different cellular compartments. Together, these studies provide a molecular basis for how NAD^+ homeostasis factors and the interacting signaling pathways confer metabolic flexibility and contribute to maintaining cell fitness and genome stability.

*To whom correspondence should be addressed: Su-Ju Lin, Department of Microbiology and Molecular Genetics, University of California, One Shields Ave, Davis, CA 95616, Tel. 530-754-6081; Fax. 530-752-9014; slin@ucdavis.edu.

We declare that no conflicts of interest exist.

Keywords

NAD^+ metabolism; NAD^+ signaling; NAD^+ homeostasis; nicotinamide riboside; yeasts

1. Introduction

Pyridine nucleotides NAD^+(H) and $NADP^+$(H) are essential coenzymes participating in many cellular redox reactions in all living systems. In addition, NAD^+ and its derivatives also function as substrates and signaling molecules in key cellular processes such as regulation of Ca^{2+} signaling, chromatin structure, DNA repair and life span (1–5). Aberrant NAD^+ metabolism has also been linked to cancer, metabolic disorders and neurodegenerative diseases such as Parkinson's and Alzheimer's disease (6–10).

Although redox reactions do not alter the total cellular NAD^+ levels, some cellular processes such as Sir2-mediated protein deacetylation, consume NAD^+; therefore constant replenishment of NAD^+ is essential for maintaining cellular fitness. Cells have developed complex interconnecting biosynthetic and signaling pathways to regulate intracellular NAD^+ levels. However, factors regulating NAD^+ metabolism and homeostasis remain unclear due to the dynamic and complex nature of the NAD^+ synthesis pathways. In this review, we summarize recent studies on the link between NAD^+ metabolism and other signaling pathways in the budding yeast *Saccharomyces cerevisiae*, and discuss our perspectives on these findings in the mechanisms underlying the regulation of NAD^+ homeostasis.

2. Overview of NAD^+ biosynthesis

Cellular NAD^+ is synthesized from a number of precursor molecules. Eukaryotes utilize the amino acid tryptophan for *de novo* biosynthesis of NAD^+. NAD^+ metabolites such as nicotinamide (Nam), nicotinic acid (NA) and nicotinamide riboside (NR) can also be salvaged and re-assimilated into NAD^+. In *S. cerevisiae*, NAD^+ is synthesized from two key intermediates nicotinic acid mononucleotide (NaMN) and nicotinamide mononucleotide (NMN) via the *de novo* and NA/Nam/NR salvaging pathways (Fig. 1). NaMN is produced by transferring the phosphoribose moiety of phosphoribosyl pyrophosphate (PRPP) to nicotinic acid (NA) or to tryptophan-derived quinolinic acid (QA), which is catalyzed by phosphoribosyltransferases Npt1 (NA phosphoribosyltransferase) and Bna6 (biosynthesis of nicotinic acid 6), respectively (11–13). QA is mainly derived from the amino acid tryptophan via five enzymatic reactions and a spontaneous cyclization (14,15). The five enzymatic reactions are mediated by Bna2 (tryptophan 2, 3-dioxygenase), Bna7 (arylformamidase), Bna1 (3-hydroxyanthranilic acid dioxygenase), Bna4 (kynurenine 3-mono oxygenase) and Bna5 (kynureninase). NA can be generated from Nam mediated by the Nam deamidase Pnc1 (pyrazineamidase and nicotinamidase) as part of the salvaging reactions. Both NA and QA can also be acquired from the environment by the NA transporter Tna1 (transporter of nicotinic acid) (16,17). *De novo* synthesis and NA/Nam salvaging pathways converge at NaMN, which is converted to deamido-NAD^+ (NaAD) by transferring the AMP moiety of ATP by Nma1 and Nma2 (NMNATs). Amidation of NaAD by Qns1 (glutamine (Q)-dependent NAD^+ synthetase) completes NAD^+ synthesis (18). NMN is produced from NR by Nrk1 (nicotinamide riboside kinase) -catalyzed

phosphorylation (19,20) and is subsequently adenylylated to NAD^+ by Nma1 and Nma2 (nicotinamide mononucleotide adenylyltransferase) (21–25). Nrk1 can also convert nicotinic acid riboside (NaR), a deamidated form of NR, to NaMN (20). Unlike higher eukaryotes including mammals, yeasts do not possess the gene coding for Nampt (Nam phosphorybosyltransferase), the enzyme that converts Nam to NMN. Instead, Nam is deamidated to NA by Pnc1 (26).

Yeast cells appear to produce NAD^+ predominantly via the NA/Nam salvage pathway during exponential growth (27) because standard growth media contain high concentrations (400 μg/l or ~ 3 μM) of niacin (NA and Nam) and budding yeast has a very efficient NA transporter Tna1 (Km for NA = ~ 1.7 μM) (17). Interestingly, although NR is an endogenous metabolite (28), standard yeast growth media do not contain NR because they do not support the growth of NAD^+ synthesis mutants defective in both *de novo* and NA/Nam salvaging (for example, the *qns1*Δ mutant). It is possible that NR is degraded during the production of components of yeast media (for example, yeast extract) (29). Unlike NA and Nam, NR is more susceptible to heat and perhaps other stresses (Kato *et al.*, unpublished results). Nevertheless, NR has been shown to be an efficient NAD^+ precursor that contributes to the NAD^+ pool and supports NAD^+-dependent reactions (19,20). NR can be acquired from the environment by the high-affinity NR transporter Nrt1 (nicotinamide riboside transporter) (Km for NR = ~ 22 μM) (30) or by salvaging intracellular intermediates. Intriguingly, yeast cells constantly release NR to the growth medium and retrieve it back to cells (28) allowing NR to traffic between intracellular and extracellular compartments. It is possible that maintaining a flexible NR/NAD^+ pool may facilitate prompt adjustments of cells in response to metabolic stresses (31). Intracellular level of NR in average wild type yeast cell is ~ 2–10 μM (28,32). Level of released NR in the growth medium varies depending on the growth conditions and genetic background. For example, in a standard late log phase cell culture in YPD (10 mL, total cell number ~3×10^9), NR in the culture is estimated to be ~0.5 μM. Salvaging endogenously produced NR has been shown to be essential for maintaining NAD^+ homeostasis and cellular fitness (28,33). An NR assimilation mutant (*nrk1*Δ*pnp1*Δ*urh1*Δ) is estimated to lose up to 0.3 mM of NR (28) and its NAD^+ level is decreased by ~50% (20).

Recent studies have identified additional NR salvaging factors connecting NR metabolism to the NA/Nam salvaging pathway (Fig. 1). The nucleosidases Pnp1 (purine nucleoside phosphorylase), Urh1 (uridine hydrolase), and Meu1 (methylthioadenosine phosphorylase, originally identified as multicopy enhancer of *UAS2*) have been shown to convert NR to Nam, or NaR to NA (20). The nucleotidases Isn1 (IMP-specific 5′-nucleotidase) and Sdt1 (suppressor of disruption of TFIIS) (33) and phosphatases Pho5 and Pho8 (28,34) have also been shown to contribute to NR metabolism by converting NMN to NR. Additional routes may exist to assist the interconversion of NAD^+ intermediates. For example, although purified recombinant Pnc1 appeared to be specific for the conversion of Nam to NA (33), a cell lysate-based study indicated that Pnc1 might also deamidate NMN and NR (28). In addition, we recently observed unexpected opposite expression patterns of functionally linked *PNC1* (Nam deamidase, converts Nam to NA) and *NPT1* (converts NA to NaMN) (Fig. 1) in late log phase. A significant up-regulation of *PNC1* gene expression was

accompanied by down-regulation of *NPT1* (Kato *et al.*, unpublished results), which contradicts the expectation that the expression levels of *NPT1* and *PNC1* are positively correlated based on the known function of these two genes. These observations suggest that Pnc1 might have additional substrates or functions, and further studies are required to explore these possibilities.

3. Crosstalk between NAD^+ metabolism and nutrient/stress sensing pathways

3.1 Sir2, calorie restriction, and NAD^+ metabolism

The Sir2 (silent information regulator 2) family proteins are highly conserved from bacteria to human (35,36). These proteins, collectively called sirtuins, harbor a conserved core domain required for its enzymatic activity. Many sirtuins have been biochemically characterized as NAD^+-dependent protein deacetylases or ADP-ribosylases (37). Sir2 was first identified as a component of the Sir1/2/3/4 silencing complexes in yeast (38,39). The Sir silencing complexes are required for the maintenance and repression of three repetitive genomic regions: telomeres, the cryptic mating type loci (*HML* and *HMR*), and the ribosomal DNA loci (40–43). Each locus employs a unique set of DNA binding factors to recruit specific Sir proteins to mediate transcriptional silencing by generating a compact chromatin configuration. Among these Sir proteins, only Sir2 is required for maintaining the silencing of all three repetitive regions. The NAD^+-dependent histone deacetylase activity of Sir2 provides a molecular mechanistic basis for Sir complex-mediated transcriptional silencing (44–46).

Sir2 homologs in yeast include Hst1, Hst2, Hst3 and Hst4 (homologous to Sir two) (35). Among those, Hst1 has the closest homology to Sir2 and has been shown to suppress silencing defects at the mating type loci of a *sir2*Δ mutant when overexpressed (35). However, it is suggested that Hst1 mediates transcriptional regulation independent of the SIR silencing complex by interacting with another transcription regulator Sum1 (47,48). Hst2 is the most abundantly expressed Sir2 homolog in yeast and accounts for most of the intracellular NAD^+-dependent deacetylase activity (45). Hst2 appears to shuttle between the nucleus and the cytoplasm to regulate rDNA and telomeric silencing (45,49–52). Hst3 and Hst4 have also been linked to the regulation of genome stability (35,53).

Overexpression (one-extra copy) of Sir2 has been shown to extend yeast replicative life span (54). In metazoa, tissue-specific overexpression of Sir2 homologs has been shown to extend life span and ameliorate functional defects associated with aging (55–60). It appears that the expression of metazoan Sir2 must be temporally regulated and controlled at a moderate level in specific tissues to exert beneficial effects (55–57,59,61). The role of Sir2 in yeast life span regulation is also complex. Yeast life span has been defined and studied in two distinct ways: replicative lifespan (RLS) measures the division potential of individual cells (62), whereas chronological lifespan (CLS) measures the rate of post-mitotic survival of a non-dividing cell population (63,64). Various longevity factors (which affect either RLS, CLS or both) have been identified in *S. cerevisiae*, and some of these factors are associated with Sir2 function. These factors include mitochondrial function (65–70), stress response/

hormesis/mitohormesis (64,66,68,71–74), the NAD^+-dependent deacetylase Sir2 family (75,76), partitioning of damaged proteins (77,78), genome stability (79–81), homeostasis of NAD^+ and other metabolic factors (1,6,31,82,83), vacuolar function (84,85), ribosome biogenesis (86), cell hypertrophy (87–89), proteostasis (90–92) and metabolites-induced toxicity (93–97). Although whether and how these factors contribute to Sir2 mediated life span extension remain debatable, it is clear that Sir2 promotes RLS under normal conditions. The role of Sir2 in CLS is less clear and in certain genetic backgrounds, deleting *SIR2* appeared to extend CLS (98), highlighting the complex role Sir2 in life span regulation.

Sir2 has also been described as a key factor that mediates the beneficial effects of calorie restriction (CR); however, its role in CR is also highly debatable. In yeast, CR is often defined as the moderate reduction of glucose concentration in the growth medium from 2% to 0.5% (1,51,99–103). This moderate CR regimen extends both RLS and CLS. Interestingly, although moderate CR largely requires the Sir2 family proteins for RLS extension (51), Sir2 appears to be dispensable for RLS extension mediated by more severe CR condition (0.05% glucose, 10X further reduction of glucose compared with moderate CR, 0.5%)(104). In fact, severe CR extends RLS even more significantly without the Sir2 family (100). This phenomenon is reminiscent of the Sir2 effect on CLS: under nutrient deprivation, deleting *SIR2* further extends CLS in certain genetic backgrounds (98). It is likely that cells respond to these two CR regimens differently. For example, severe CR may additionally trigger gluconeogenesis, since gluconeogenesis is activated upon glucose starvation. It has been suggested that gluconeogenesis is induced in the *sir2*Δ mutant and therefore contributes to CLS extension (98). Supporting this, a key gluconeogenesis enzyme Pck1 was found to be a substrate of Sir2. In *sir2*Δ mutant, Pck1 remains active and gluconeogenesis is promoted (105). On the other hand, age-enhanced gluconeogenesis has been shown to shorten CLS and Hst3 and Hst4 down-regulate age-enhanced gluconeogenesis (106). In addition, human Sirt1 has been reported to deacetylate PGC1-α, which leads to induction of gluconeogenesis (107). Together, these studies demonstrate the complex roles of gluconeogenesis and Sir2 in CR, which are not surprising for metabolic longevity factors. In addition to the Sir2 family, CR is suggested to function through reducing the activities of conserved nutrient-sensing pathways to extend life span. Such pathways include the Ras-cAMP/PKA (cyclic-AMP activated protein kinase A) pathway, the nutrient-responsive Sch9/AKT (homolog of mammalian S6K kinases) and TOR (target of rapamycin) kinase pathways (Fig. 2) (108,109). CR also induces the shunting of carbon metabolism from fermentation towards the mitochondrial TCA cycle, and a concomitant increase of respiration (110). The Sir2 family proteins have been shown to interact with or modulate the activity of these CR-associated longevity factors. We await future studies to further detail the mechanisms underlying the complexity of Sir2 functions in CR.

NAD^+ and its derivatives are important Sir2 regulators. NAD^+ levels affect Sir2 activity and consequently the downstream events. Mutations that cause deficiency in NAD^+ production affect Sir2-mediated processes. Deleting the *NPT1* gene significantly reduces NAD^+ levels and abolishes Sir2-mediated silencing (45) and CR-mediated life span extension (1). Nam is a by-product generated during Sir2-mediated deacetylation, which is also a potent non-competitive inhibitor of Sir2 (111–113). The nicotinamidase Pnc1 is responsible for the

clearance of Nam by converting it to nicotinic acid (NA) (26). Increasing Nam concentration in the growth medium or blocking Nam clearance by deleting the *PNC1* reduced Sir2-mediated silencing and shortened life span (Fig. 2) (111,114), suggesting that preventing Nam accumulation is important for Sir2 function. NADH was reported to be a competitive inhibitor of Sir2, and lowering the NADH level by genetic modifications or CR increased Sir2-mediated silencing and life span (115). However, NADH is a weak inhibitor of Sir2, and reported *in vivo* NADH levels are too low to inhibit Sir2 activity (113). Therefore, it is possible that intracellular compartmentalization of NAD^+ and NADH and/or specific protein-protein interactions create locally high NAD^+/NADH ratios, thereby activating Sir2 *in vivo*. It has been suggested that the affinity/sensitivity of Sir2 towards its substrates and inhibitors varied when Sir2 was in complex with different interacting partners (116). Interestingly, high concentrations of NMN could inhibit Sir2 activity *in vitro* (IC_{50} = ~ 3 mM) (113). However, this inhibition is unlikely to be physiologically relevant considering low intracellular concentration of NMN (~ 60 μM) (32).

In addition to being regulated by NAD^+ and its derivatives, both Sir2 and Hst1 have also been shown to regulate NAD^+ metabolism. It has been reported that *de novo* NAD^+ synthesis is regulated by Hst1-mediated repression and that deletion of *HST1* raises the steady-state level of NAD^+ (117). Hst1 has also been shown to repress the expression of NR transporter Nrt1 (118). Our recent studies suggested that Sir2 modulates the flux of NR salvage (28). The Sir2 family has been proposed to support the NA/Nam salvage pathway via producing Nam in deacetylation reactions (Fig. 1 and Fig. 2) (82). However, the precise role of Sir2 family proteins in NAD^+ metabolism remained unclear. Increased NR release observed in the *sir2*Δ mutant suggested a specific role of Sir2 in NR salvage (28). It is possible that Sir2 helps to preserve the NAD^+ pool by decreasing the pyridine nucleotide flux into the NR branch. Indeed, deleting *SIR2* in cells lacking NR assimilation activity further decreased the size of the NAD^+ pool (28). We await future studies to determine the molecular and biochemical roles of the Sir2 family in NR and NAD^+ metabolism.

3.2 The *PHO* pathway and NAD^+ metabolism

Our recent studies have uncovered a link between the regulation of NAD^+ metabolism and phosphate signaling (34). In *S. cerevisiae*, phosphate homeostasis is regulated by the phosphate-responsive signaling pathway (*PHO* pathway), which monitors and responds to changes in the phosphate availability (119,120). The *PHO* pathway consists of regulatory components and downstream target genes whose expression is regulated by transcription factors Pho4 and Pho2. Activation of *PHO* pathway is influenced by intracellular phosphate (Pi) levels and phosphorylation state of Pho4 determines the gene expression of the downstream targets. In high Pi conditions, the cyclin-cyclin-dependent kinase (cyclin-CDK) complex Pho80-Pho85 phosphorylates Pho4, which is then exported out of the nucleus. Upon Pi starvation, the CDK inhibitor Pho81 binds the Pho80-Pho85 complex and inhibits Pho4 phosphorylation, allowing Pho4 to translocate to the nucleus and activate the expression of its target genes (119,120). Pho4-induced genes include those encoding repressible acid and alkaline phosphatases Pho5 and Pho8, high affinity phosphate transporters Pho84 and Pho89, and vacuolar proteins that mediate the utilization of stored polyphosphates (121).

We previously reported that production of NR correlates with activation of *PHO* signaling (34). Intracellular NR level is increased under conditions that activate the *PHO* pathway (such as in low Pi growth media and deletion of *PHO84*), and is decreased in cells defective in *PHO* signaling (such as the *pho4*Δ mutant). Increased NR production under high *PHO* activity conditions is largely due to the activation of the vacuolar phosphatase Pho8, since deletion of *PHO8* gene significantly decreases intracellular NR level (34). Our studies also demonstrated that utilization of extracellular NMN requires prior conversion to NR mediated by the *PHO*-regulated periplasmic phosphatase Pho5 (31). The cytosolic nucleotidase Sdt1, which plays an important role in NR production (by hydrolyzing NMN) (33), has also been suggested to be under *PHO* regulation (122). Together, these studies show evidence for *PHO* regulation of several components of NAD^+ metabolism in yeast. Some of these studies may be extrapolated to understanding NR/NAD^+ metabolism in mammalian cells. For example, "conversion to NR" (and other smaller NAD^+ intermediates) may represent a strategy for the transport and assimilation of large impermeable NAD^+ precursors. Supplementing NAD^+ precursors and intermediates has been shown to replenish NAD^+ level and ameliorate functional defects associated with abnormal NAD^+ metabolism in mammalian models (59,123–125). Because the structures of many NAD^+ precursors are unfavorable for direct diffusion across the cell membrane, either specific transporters or extracellular catabolizing enzymes would be required to utilize exogenous NAD^+ precursors. In a study using a system where Nam utilization is blocked (by Nampt inhibitors), it was suggested that extracellular NAD^+ and NMN need to be converted to NR for cells to utilize them. Interfering with the enzymatic activities that mediate the degradation of extracellular NAD^+ to NMN, or NMN to NR reduced the ability of cells to synthesize NAD^+. Although the transport mechanism of NR has not been characterized in mammalian cells, pharmacological inhibition of plasma membrane nucleoside transporters reduced intracellular NAD^+ production (126). Supporting this model, a recent study showed that CD73, an extracellular (periplasmic) nucleotidase orthologous to *H. influenza* NadN, could convert NAD^+ and NMN to NR (127). In addition, it has been suggested that NR is circulated in the peripheral bloodstream in mammals (128). To date, the molecular aspects of phosphate signaling in mammalian cells remain unclear. Interesting future studies would be to determine whether NAD^+ metabolism is also linked to phosphate signaling in higher eukaryotes.

In addition to regulating NAD^+ metabolism, the *PHO* pathway was suggested to respond to intracellular NaMN levels (34). Npt1 is the major enzyme that mediates the production of NaMN from NA. Increased activity of a *PHO*-regulated acid phosphatase Pho5 was observed specifically in *npt1*Δ mutant, suggesting that depletion of NaMN leads to *PHO* activation (34). Mechanisms underlying the cross-regulation between *PHO* signaling and NAD^+ metabolism remain unclear. The coupling of these two pathways may render a more efficient metabolic support under specific conditions. For example, the phosphate moiety of NMN is a putative target for phosphate scavenging during Pi limitation. Moreover, since NaMN is an important intermediate for NAD^+ biosynthesis, low level of NaMN might reflect impaired NAD^+ biosynthesis and lead to activation of an alternative NAD^+ salvage route. Since NR-mediated NAD^+ synthesis requires phosphate (in the form of ATP), a

coordinated activation of the *PHO* pathway is expected in supporting NAD^+ synthesis and homeostasis.

3.3 Other *PHO*-interacting nutrient/stress sensing pathways and their potential roles in NAD^+ metabolism

Research in recent years has also connected *PHO* signaling to other nutrient sensing pathways (Fig. 2). For example, it has been shown that, after prolonged Pi starvation, the repletion of Pi to cells leads to degradation of the high-affinity phosphate transporter Pho84 via the activation of protein kinase A (PKA) (129–133), demonstrating a connection between the glucose and phosphate signaling pathways. These studies also support a role for Pho84 as a Pi transceptor, which can sense as well as transport Pi. Other components in the *PHO* pathway have also been associated with glucose metabolism (134,135). In addition, *PHO* signaling and Sch9/Akt and TOR pathways appear to converge on Rim15 to regulate stress response transcription factors such as Msn2 and Msn4 (103,136,137). However, these pathways also affect different targets and often synergistically or oppositely regulate specific targets (138). Complex crosstalk between these pathways may allow cells to quickly adapt to environmental changes.

How may these nutrient sensing pathways regulate NAD^+ metabolism? The stress-responsive transcriptional factors Msn2 and Msn4 have been shown to regulate *PNC1* expression, and are required for CR-mediated life span extension (139). Increased expression of *PNC1* was also observed under various mild stresses such as glucose or amino acid restriction, and heat or salt stresses (140). In addition, correlation between mRNA mistranslation and *PNC1* up-regulation has been shown (141). Given that mild stresses can mediate life span extension (99,140,142) and that CR induces mild stress signals (73), it is possible that Pnc1 is a key factor that connects stress signals (including CR) to the regulation of Sir2 (143) as well as NAD^+ metabolism (Fig. 2). Interestingly, Nampt (nicotinamide phosphoribosyltransferase), the functional homolog of Pnc1 in higher eukaryotes, is also responsive to stresses and suggested to be the mediator of CR-induced benefits (144,145)

Considering Nam is a potent Sir2 inhibitor, mechanisms to control Nam concentration would be key to maintain Sir2 activity. In yeast, Nam clearance is also achieved by methylation catalyzed by the Nam methyltransferase Nnt1, a homolog of human NNMT (N-nicotinamide methyltransferase) (115,140). *NNT1* overexpression was shown to rescue a *PNC1* null mutation and restore CR-mediated life span extension in this genetic background (115). A recent study in *C. elegans* suggests that methylated Nam can induce a transient hormetic response, which leads to life span extension (146). The worm amine N-methyltransferase ANMT-1 contributes to the production of N-methylnicotinamide. Methylated Nam is a substrate of aldehyde oxidase, an H_2O_2-generating enzyme thereby inducing transient increase of ROS (reactive oxygen species) signal. Moreover, specific NAD^+ synthesis components have also been shown to play a role in other cellular processes. For example, NMNAT family proteins were reported to affect cellular functions independent of their enzymatic function. *Salmonella* NadR is a multi-functional NMNAT with a role in transcriptional regulation (147). *Drosophila* NMNAT was shown to possess

chaperone activity that contributes to axonal protection (7). In *S. cerevisiae*, Nma1 and Nma2 have been shown to alleviate proteotoxicity in yeast models of proteinopathies (148). Overall, these studies have demonstrated that specific NAD^+ intermediates as well as NAD^+ metabolism components also play a role in other cellular processes, further highlighting the importance of NAD^+ homeostasis factors.

4. Intracellular compartmentalization of the NAD^+ intermediate pools

4.1 Mitochondrial compartmentalization of NAD^+ (H)

In yeast, NAD^+ synthesis seems to occur mainly in the cytosol. It remains unclear how NAD^+ and NAD^+ derivatives are transported in and out of various intracellular compartments. Transport of NAD^+ into the mitochondria can be mediated by the NAD^+ carrier proteins encoded by *NDT1* and *NDT2* (149). Our understanding of mitochondrial NAD^+(H) homeostasis mostly derives from studying the NAD^+/NADH shuttle systems. Since the mitochondrial inner membrane is impermeable to NAD^+ and NADH, the NADH shuttle systems function to move small permeable redox equivalents of NAD^+ and NADH across the mitochondrial membrane to balance the NAD^+/NADH ratio between the mitochondrial and the cytosolic/nuclear pools (150,151). For example, respiration-induced increase in NAD^+/NADH ratio in the mitochondria can be transmitted to the cytosol by the malate-aspartate shuttle (Fig. 3). In yeast, CR induces a metabolic shift from fermentation to mitochondrial respiration (110) and a concomitant increase in the NAD^+/NADH ratio (115). Because respiration produces NAD^+ from NADH and NADH can function as a competitive inhibitor of Sir2 activity (115), it has been suggested that increased NAD^+/NADH ratio may activate Sir2 during CR. Supporting this model, genetic manipulations that decrease NADH levels are shown to increase Sir2 activity and extend life span (110,115). In addition, deleting NADH shuttle components abolishes CR-mediated life span extension whereas overexpressing NADH shuttle components extends life span (101). This model has remained controversial because reported *in vivo* NADH levels are probably too low to inhibit Sir2 activity (113). It is possible that intracellular compartmentalization of NAD^+ and NADH creates locally high NAD^+/NADH ratios thereby activating Sir2 *in vivo.* It is also possible that when Sir2 forms specific protein-protein interactions with different partners, the affinity/sensitivity of Sir2 toward its substrates and inhibitors may vary (116). Another model (not mutually exclusive) is that the level of Nam, a much more potent Sir2 inhibitor, is decreased by CR (111) leading to Sir2 activation. CR has been shown to increase the expression of *PNC1* (a Nam deamidase that converts Nam to NA) (140), which is expected to decrease Nam level, leading to Sir2 activation. However, since Nam is also a key NAD^+ precursor in the NA/Nam salvage pathway, it is unclear whether CR-induced Sir2 activation is due to increased NAD^+ availability, reduced Nam or both.

In mammals, CR has also been reported to affect NAD^+/NADH ratio in a tissue-specific manner, and these changes correlate with the levels of SIRT1 expression/activity (152,153). Molecular mechanisms underling CR-induced changes in the NAD^+/NADH ratios and the subsequent events remain to be elucidated. In yeast, CR increases NAD^+/NADH ratio by decreasing the NADH level (115), whereas in mouse, CR triggers different responses in different tissues (152,153). In muscle and white adipose tissues, the increase in NAD^+/

NADH ratios appeared to be due to increased NAD^+ levels (152). In liver, CR actually decreases NAD^+/NADH ratio by increasing the NADH level (152). In pancreas, fasting decreases NAD^+/NADH ratio by decreasing NAD^+ level (153). Despite the complexity of the regulation of NAD^+/NADH ratios and Sir2 activities as well as the controversies whether Sir2 is activated by CR, there is no doubt that sufficient NAD^+ level (i.e. functional NAD^+ biosynthesis) is required for Sir2 activity and certain CR-induced beneficial effects. Functional NADH shuttles may confer metabolic flexibility to support multiple CR-induced molecular changes. In mammals, impairments of mitochondrial metabolism and NADH shuttles have also been implicated in age-associated diseases such as diabetes (154,155). Overall, these studies suggest that the NADH shuttles play important roles in mitochondrial metabolism and metabolic fitness by regulating NAD^+ homeostasis. Identification of additional mitochondrial NAD^+ homeostasis factors including the pyridine nucleotide transporters will provide insights into the understanding of the roles of mitochondria in NAD^+ homeostasis.

4.2 Vacuolar and cytosolic compartmentalization of NMN and NR

Our recent studies suggest that NAD^+ metabolism is connected to the *PHO* pathway. In particular, *PHO*-activated Pho8 and Pho5 contribute directly to NR production by releasing phosphate from NMN (28). Since Pho8 is a vacuolar membrane protein whose activation requires a vacuolar peptidase (156), we speculate that the vacuole is a cellular compartment for NR production and storage (Fig. 3). Supporting this possibility, our previous genetic screens searching for mutants that show altered NR release have identified factors involved in vacuolar ATP synthase (v-ATPase) assembly (34). These v-ATPase-related factors are known to function in establishing and maintaining the acidity of the vacuolar matrix. We found that 12 mutants harboring deficiencies in the regulation or the structure of v-ATPase complexes released more NR (2- to 3-fold higher than the wild type). Furthermore, 19 mutants with deficiencies in vesicle-mediated transport also manifested increased NR release (2- to 3-fold higher than the wild type). Collectively, these results reinforced the idea that the vacuole and vesicle-mediated transport might play a role in NR metabolism and homeostasis.

If the vacuole is a cellular compartment for NR production and storage, how do cells salvage vacuolar NR to synthesize NAD^+ in the cytosol? Fun26 may function to balance NR between the cytosolic and the vacuolar pools, enabling assimilation of NR originating from the vacuole into cytosolic NAD^+ synthesis (Fig. 3) (34,157,158). Fun26 is the only yeast homolog of the human equilibrative nucleoside transporter (hENT) protein family, which mediates bi-directional transport of specific nucleosides across the plasma membrane and intracellular membranes (159–161). In yeast, the Fun26 protein was reported to have a minor role in NR transport relative to Nrt1 (30). However, the *fun26*Δ mutant displayed a level of NR release increase (3- to 4-fold higher than the wild type) comparable to that seen for the *nrt1*Δ mutant (34). In addition, a significant increase in intracellular NR level was also observed in the *fun26*Δ mutant (4- to 5-fold higher than the wild type) but not in the *nrt1*Δ mutant (similar to the wild type) (34). Therefore Fun26 may play a significant role in balancing the NR pools produced and stored in the vacuole and the NR pools used for NAD^+ synthesis in the cytosol.

These studies also suggest that for each individual cell, the level of released NR reflects the size of its dynamic cytosolic NR pool, and that the steady state NR level (determined in total cell extract) reflects the size of the stored pool (Fig. 3) (34). Supporting this idea, deletions of genes encoding cytosolic nucleotidases Isn1 and Sdt1 (33) significantly decreased NR release (~40 % decrease) (33,162) but only slightly affected intracellular NR level (~15 % decrease) (34,162). Conversely, deleting *PHO8* largely decreased intracellular NR level (50–70 % decrease) but only slightly affected the level of NR release (20–30 % decrease) (34,162). To date, the mechanisms of NR uptake and its transport between cellular compartments in higher eukaryotes are still unknown. Four Fun26-related human ENT (equilibrative nucleoside transporter) proteins have been characterized (159). Given the role of Fun26 in NR metabolism in yeast, it is possible that the plasma membrane-localized hENT1 and hENT2 and lysosome membrane-resided hENT3 also participate in NR homeostasis and NAD^+ metabolism in human. One additional potential NAD^+ homeostasis compartment is the peroxisome. Peroxisomal Npy1 has been shown to function as a NAD^+(H) pyrophosphatase (or Nudix hydrolase) which could produce NMN(H) from NAD^+(H) (163) and contribute to NAD^+ homeostasis. Future studies on the identification of novel NAD^+ homeostasis factors will further our understanding of how these compartments and associated factors regulate NAD^+ metabolism and cell functions.

5. Conclusion and Perspectives

Intracellular concentrations of many of the NAD^+ intermediates are maintained at low levels (32), which is characteristic of signaling molecules. NR and NMN, similar to other NAD^+ intermediates, may function as signaling molecules to regulate NAD^+ homeostasis or other cellular processes. We have previously discovered that low NaMN level is associated with the activation of the *PHO* pathway (34). Moreover, nicotinic acid adenine dinucleotide phosphate (NAADP) has been shown to function as a signaling molecule to regulate calcium homeostasis in variety of organisms (164,165). In addition to functioning as signaling molecules, high concentrations of intracellular NAD^+ intermediates may be unfavorable for certain cellular processes. For example, NAD^+-dependent DNA ligase in bacteria is inhibited by NMN and it is suggested that NMN deamidase contributes to maintaining small intracellular NMN pool (166). As discussed earlier, Nam is known as an inhibitor of Sir2 and clearance of Nam is critical for maintaining Sir2 activity and life span (114). Nam clearance is facilitated by a Nam deamidase Pnc1 in yeast or Nampt, a Nam phosphoribosyltransferase in mammals (111,167). Also, methylation of Nam offers beneficial effects by clearing Nam and providing stress resistance (146). Studies in yeast suggest that cells may constantly convert NMN to NR, which is more mobile and can be readily excreted, stored or re-assimilated (28,34). Ohashi *et al*. showed that quinolinic acid (QA) is also produced and excreted like NR (16). Thus, it is possible that pyridine nucleotides and their metabolites are involved in a variety of cellular processes and balancing their concentrations would be critical for the regulation of these processes. Future studies on the identification and characterization of additional signaling factors that regulate and/or crosstalk with NAD^+ homeostasis are highly anticipated. Overall, these studies have contributed to the understanding of the complex NAD^+ homeostasis pathways, and may also

provide insights into the underlying mechanisms of diseases related to defects in NAD^+ metabolism.

Acknowledgments

We are grateful to the researchers whose work provided the basis for this review. We thank Felicia Tsang and members of the Lin laboratory for discussions and suggestions. The Lin laboratory is supported by the National Institutes of Health, NIGMS (GM102297).

References

1. Lin SJ, Defossez PA, Guarente L. Requirement of NAD and SIR2 for life-span extension by calorie restriction in Saccharomyces cerevisiae. Science. 2000; 289:2126–2128. [PubMed: 11000115]
2. Lin SJ, Guarente L. Nicotinamide adenine dinucleotide, a metabolic regulator of transcription, longevity and disease. Curr Opin Cell Biol. 2003; 15:241–246. [PubMed: 12648681]
3. Rusche LN, Kirchmaier AL, Rine J. The establishment, inheritance, and function of silenced chromatin in Saccharomyces cerevisiae. Annu Rev Biochem. 2003; 72:481–516. [PubMed: 12676793]
4. Burkle A. Poly(ADP-ribose). The most elaborate metabolite of NAD+ FEBS J. 2005; 272:4576–4589. [PubMed: 16156780]
5. Chini EN. CD38 as a regulator of cellular NAD: a novel potential pharmacological target for metabolic conditions. Curr Pharm Des. 2009; 15:57–63. [PubMed: 19149603]
6. Belenky P, Bogan KL, Brenner C. NAD+ metabolism in health and disease. Trends Biochem Sci. 2007; 32:12–19. [PubMed: 17161604]
7. Zhai RG, Zhang F, Hiesinger PR, Cao Y, Haueter CM, Bellen HJ. NAD synthase NMNAT acts as a chaperone to protect against neurodegeneration. Nature. 2008; 452:887–891. [PubMed: 18344983]
8. Khan JA, Tao X, Tong L. Molecular basis for the inhibition of human NMPRTase, a novel target for anticancer agents. Nature structural & molecular biology. 2006; 13:582–588.
9. Imai S, Yoshino J. The importance of NAMPT/NAD/SIRT1 in the systemic regulation of metabolism and ageing. Diabetes Obes Metab. 2013; 15(Suppl 3):26–33. [PubMed: 24003918]
10. Mouchiroud L, Houtkooper RH, Auwerx J. NAD(+) metabolism: a therapeutic target for age-related metabolic disease. Crit Rev Biochem Mol Biol. 2013; 48:397–408. [PubMed: 23742622]
11. Panozzo C, Nawara M, Suski C, Kucharczyka R, Skoneczny M, Becam AM, Rytka J, Herbert CJ. Aerobic and anaerobic NAD+ metabolism in Saccharomyces cerevisiae. FEBS Lett. 2002; 517:97–102. [PubMed: 12062417]
12. Preiss J, Handler P. Biosynthesis of diphosphopyridine nucleotide. II. Enzymatic aspects. J Biol Chem. 1958; 233:493–500. [PubMed: 13563527]
13. Preiss J, Handler P. Biosynthesis of diphosphopyridine nucleotide. I. Identification of intermediates. J Biol Chem. 1958; 233:488–492. [PubMed: 13563526]
14. Bender DA. Biochemistry of tryptophan in health and disease. Mol Aspects Med. 1983; 6:101–197. [PubMed: 6371429]
15. Wogulis M, Chew ER, Donohoue PD, Wilson DK. Identification of formyl kynurenine formamidase and kynurenine aminotransferase from Saccharomyces cerevisiae using crystallographic, bioinformatic and biochemical evidence. Biochemistry. 2008; 47:1608–1621. [PubMed: 18205391]
16. Ohashi K, Kawai S, Murata K. Secretion of quinolinic acid, an intermediate in the kynurenine pathway, for utilization in NAD+ biosynthesis in the yeast Saccharomyces cerevisiae. Eukaryot Cell. 2013; 12:648–653. [PubMed: 23457190]
17. Llorente B, Dujon B. Transcriptional regulation of the Saccharomyces cerevisiae DAL5 gene family and identification of the high affinity nicotinic acid permease TNA1 (YGR260w). FEBS Lett. 2000; 475:237–241. [PubMed: 10869563]

18. Bieganowski P, Pace HC, Brenner C. Eukaryotic NAD+ synthetase Qns1 contains an essential, obligate intramolecular thiol glutamine amidotransferase domain related to nitrilase. J Biol Chem. 2003; 278:33049–33055. [PubMed: 12771147]
19. Bieganowski P, Brenner C. Discoveries of nicotinamide riboside as a nutrient and conserved NRK genes establish a Preiss-Handler independent route to NAD+ in fungi and humans. Cell. 2004; 117:495–502. [PubMed: 15137942]
20. Belenky P, Racette FG, Bogan KL, McClure JM, Smith JS, Brenner C. Nicotinamide riboside promotes Sir2 silencing and extends lifespan via Nrk and Urh1/Pnp1/Meu1 pathways to NAD+ Cell. 2007; 129:473–484. [PubMed: 17482543]
21. Natalini P, Ruggieri S, Raffaelli N, Magni G. Nicotinamide mononucleotide adenylyltransferase. Molecular and enzymatic properties of the homogeneous enzyme from baker's yeast. Biochemistry. 1986; 25:3725–3729. [PubMed: 3013296]
22. Garavaglia S, D'Angelo I, Emanuelli M, Carnevali F, Pierella F, Magni G, Rizzi M. Structure of human NMN adenylyltransferase. A key nuclear enzyme for NAD homeostasis. J Biol Chem. 2002; 277:8524–8530. [PubMed: 11751893]
23. Emanuelli M, Amici A, Carnevali F, Pierella F, Raffaelli N, Magni G. Identification and characterization of a second NMN adenylyltransferase gene in Saccharomyces cerevisiae. Protein expression and purification. 2003; 27:357–364. [PubMed: 12597897]
24. Magni G, Amici A, Emanuelli M, Orsomando G, Raffaelli N, Ruggieri S. Structure and function of nicotinamide mononucleotide adenylyltransferase. Curr Med Chem. 2004; 11:873–885. [PubMed: 15078171]
25. Emanuelli M, Carnevali F, Lorenzi M, Raffaelli N, Amici A, Ruggieri S, Magni G. Identification and characterization of YLR328W, the Saccharomyces cerevisiae structural gene encoding NMN adenylyltransferase. Expression and characterization of the recombinant enzyme. FEBS Lett. 1999; 455:13–17. [PubMed: 10428462]
26. Ghislain M, Talla E, Francois JM. Identification and functional analysis of the Saccharomyces cerevisiae nicotinamidase gene, PNC1. Yeast. 2002; 19:215–224. [PubMed: 11816029]
27. Sporty J, Lin SJ, Kato M, Ognibene T, Stewart B, Turteltaub K, Bench G. Quantitation of NAD+ biosynthesis from the salvage pathway in Saccharomyces cerevisiae. Yeast. 2009; 26:363–369. [PubMed: 19399913]
28. Lu SP, Kato M, Lin SJ. Assimilation of endogenous nicotinamide riboside is essential for calorie restriction-mediated life span extension in Saccharomyces cerevisiae. J Biol Chem. 2009; 284:17110–17119. [PubMed: 19416965]
29. Reed, G.; Nagodawithana, TW. Yeast technology. Van Nostrand Reinhold; New York: 1991.
30. Belenky PA, Moga TG, Brenner C. Saccharomyces cerevisiae YOR071C encodes the high affinity nicotinamide riboside transporter Nrt1. J Biol Chem. 2008; 283:8075–8079. [PubMed: 18258590]
31. Lu SP, Lin SJ. Regulation of yeast sirtuins by NAD(+) metabolism and calorie restriction. Biochim Biophys Acta. 2010; 1804:1567–1575. [PubMed: 19818879]
32. Evans C, Bogan KL, Song P, Burant CF, Kennedy RT, Brenner C. NAD+ metabolite levels as a function of vitamins and calorie restriction: evidence for different mechanisms of longevity. BMC Chem Biol. 2010; 10:2. [PubMed: 20175898]
33. Bogan KL, Evans C, Belenky P, Song P, Burant CF, Kennedy R, Brenner C. Identification of Isn1 and Sdt1 as glucose- and vitamin-regulated nicotinamide mononucleotide and nicotinic acid mononucleotide [corrected] 5′-nucleotidases responsible for production of nicotinamide riboside and nicotinic acid riboside. J Biol Chem. 2009; 284:34861–34869. [PubMed: 19846558]
34. Lu SP, Lin SJ. Phosphate-responsive signaling pathway is a novel component of NAD+ metabolism in Saccharomyces cerevisiae. J Biol Chem. 2011; 286:14271–14281. [PubMed: 21349851]
35. Brachmann CB, Sherman JM, Devine SE, Cameron EE, Pillus L, Boeke JD. The SIR2 gene family, conserved from bacteria to humans, functions in silencing, cell cycle progression, and chromosome stability. Genes Dev. 1995; 9:2888–2902. [PubMed: 7498786]
36. Frye RA. Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins. Biochemical and biophysical research communications. 2000; 273:793–798. [PubMed: 10873683]

37. North BJ, Verdin E. Sirtuins: Sir2-related NAD-dependent protein deacetylases. Genome biology. 2004; 5:224. [PubMed: 15128440]
38. Ivy JM, Klar AJ, Hicks JB. Cloning and characterization of four SIR genes of Saccharomyces cerevisiae. Mol Cell Biol. 1986; 6:688–702. [PubMed: 3023863]
39. Rine J, Herskowitz I. Four genes responsible for a position effect on expression from HML and HMR in Saccharomyces cerevisiae. Genetics. 1987; 116:9–22. [PubMed: 3297920]
40. Hecht A, Strahl-Bolsinger S, Grunstein M. Spreading of transcriptional repressor SIR3 from telomeric heterochromatin. Nature. 1996; 383:92–96. [PubMed: 8779721]
41. Strahl-Bolsinger S, Hecht A, Luo K, Grunstein M. SIR2 and SIR4 interactions differ in core and extended telomeric heterochromatin in yeast. Genes Dev. 1997; 11:83–93. [PubMed: 9000052]
42. Smith JS, Boeke JD. An unusual form of transcriptional silencing in yeast ribosomal DNA. Genes Dev. 1997; 11:241–254. [PubMed: 9009206]
43. Moazed D. Common themes in mechanisms of gene silencing. Mol Cell. 2001; 8:489–498. [PubMed: 11583612]
44. Imai S, Armstrong CM, Kaeberlein M, Guarente L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature. 2000; 403:795–800. [PubMed: 10693811]
45. Smith JS, Brachmann CB, Celic I, Kenna MA, Muhammad S, Starai VJ, Avalos JL, Escalante-Semerena JC, Grubmeyer C, Wolberger C, Boeke JD. A phylogenetically conserved NAD+-dependent protein deacetylase activity in the Sir2 protein family. Proc Natl Acad Sci U S A. 2000; 97:6658–6663. [PubMed: 10841563]
46. Landry J, Sutton A, Tafrov ST, Heller RC, Stebbins J, Pillus L, Sternglanz R. The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. Proc Natl Acad Sci U S A. 2000; 97:5807–5811. [PubMed: 10811920]
47. Sutton A, Heller RC, Landry J, Choy JS, Sirko A, Sternglanz R. A novel form of transcriptional silencing by Sum1-1 requires Hst1 and the origin recognition complex. Mol Cell Biol. 2001; 21:3514–3522. [PubMed: 11313477]
48. Xie J, Pierce M, Gailus-Durner V, Wagner M, Winter E, Vershon AK. Sum1 and Hst1 repress middle sporulation-specific gene expression during mitosis in Saccharomyces cerevisiae. EMBO J. 1999; 18:6448–6454. [PubMed: 10562556]
49. Perrod S, Cockell MM, Laroche T, Renauld H, Ducrest AL, Bonnard C, Gasser SM. A cytosolic NAD-dependent deacetylase, Hst2p, can modulate nucleolar and telomeric silencing in yeast. EMBO J. 2001; 20:197–209. [PubMed: 11226170]
50. Halme A, Bumgarner S, Styles C, Fink GR. Genetic and epigenetic regulation of the FLO gene family generates cell-surface variation in yeast. Cell. 2004; 116:405–415. [PubMed: 15016375]
51. Lamming DW, Latorre-Esteves M, Medvedik O, Wong SN, Tsang FA, Wang C, Lin SJ, Sinclair DA. HST2 mediates SIR2-independent life-span extension by calorie restriction. Science. 2005; 309:1861–1864. [PubMed: 16051752]
52. Wilson JM, Le VQ, Zimmerman C, Marmorstein R, Pillus L. Nuclear export modulates the cytoplasmic Sir2 homologue Hst2. EMBO Rep. 2006; 7:1247–1251. [PubMed: 17110954]
53. Celic I, Masumoto H, Griffith WP, Meluh P, Cotter RJ, Boeke JD, Verreault A. The sirtuins hst3 and Hst4p preserve genome integrity by controlling histone h3 lysine 56 deacetylation. Curr Biol. 2006; 16:1280–1289. [PubMed: 16815704]
54. Kaeberlein M, McVey M, Guarente L. The SIR2/3/4 complex and SIR2 alone promote longevity in Saccharomyces cerevisiae by two different mechanisms. Genes Dev. 1999; 13:2570–2580. [PubMed: 10521401]
55. Viswanathan M, Guarente L. Regulation of Caenorhabditis elegans lifespan by sir-2.1 transgenes. Nature. 2011; 477:E1–2. [PubMed: 21938026]
56. Hoffmann J, Romey R, Fink C, Yong L, Roeder T. Overexpression of Sir2 in the adult fat body is sufficient to extend lifespan of male and female Drosophila. Aging (Albany NY). 2013; 5:315–327. [PubMed: 23765091]
57. Guarente L. Calorie restriction and sirtuins revisited. Genes Dev. 2013; 27:2072–2085. [PubMed: 24115767]
58. Herskovits AZ, Guarente L. Sirtuin deacetylases in neurodegenerative diseases of aging. Cell Res. 2013; 23:746–758. [PubMed: 23689277]

59. Imai SI, Guarente L. NAD and sirtuins in aging and disease. Trends Cell Biol. 2014
60. Chang HC, Guarente L. SIRT1 and other sirtuins in metabolism. Trends Endocrinol Metab. 2014; 25:138–145. [PubMed: 24388149]
61. Burnett C, Valentini S, Cabreiro F, Goss M, Somogyvari M, Piper MD, Hoddinott M, Sutphin GL, Leko V, McElwee JJ, Vazquez-Manrique RP, Orfila AM, Ackerman D, Au C, Vinti G, Riesen M, Howard K, Neri C, Bedalov A, Kaeberlein M, Soti C, Partridge L, Gems D. Absence of effects of Sir2 overexpression on lifespan in C. elegans and Drosophila. Nature. 2011; 477:482–485. [PubMed: 21938067]
62. Mortimer RK, Johnston JR. Life span of individual yeast cells. Nature. 1959; 183:1751–1752. [PubMed: 13666896]
63. Fabrizio P, Longo VD. The chronological life span of Saccharomyces cerevisiae. Aging Cell. 2003; 2:73–81. [PubMed: 12882320]
64. Longo VD, Fabrizio P. Chronological Aging in Saccharomyces cerevisiae. Subcell Biochem. 2012; 57:101–121. [PubMed: 22094419]
65. Barros MH, Bandy B, Tahara EB, Kowaltowski AJ. Higher respiratory activity decreases mitochondrial reactive oxygen release and increases life span in Saccharomyces cerevisiae. J Biol Chem. 2004; 279:49883–49888. [PubMed: 15383542]
66. Bonawitz ND, Chatenay-Lapointe M, Pan Y, Shadel GS. Reduced TOR signaling extends chronological life span via increased respiration and upregulation of mitochondrial gene expression. Cell Metab. 2007; 5:265–277. [PubMed: 17403371]
67. Scheckhuber CQ, Erjavec N, Tinazli A, Hamann A, Nystrom T, Osiewacz HD. Reducing mitochondrial fission results in increased life span and fitness of two fungal ageing models. Nat Cell Biol. 2007; 9:99–105. [PubMed: 17173038]
68. Ocampo A, Liu J, Schroeder EA, Shadel GS, Barrientos A. Mitochondrial respiratory thresholds regulate yeast chronological life span and its extension by caloric restriction. Cell Metab. 2012; 16:55–67. [PubMed: 22768839]
69. Veatch JR, McMurray MA, Nelson ZW, Gottschling DE. Mitochondrial dysfunction leads to nuclear genome instability via an iron-sulfur cluster defect. Cell. 2009; 137:1247–1258. [PubMed: 19563757]
70. Erjavec N, Bayot A, Gareil M, Camougrand N, Nystrom T, Friguet B, Bulteau AL. Deletion of the mitochondrial Pim1/Lon protease in yeast results in accelerated aging and impairment of the proteasome. Free Radic Biol Med. 2013; 56:9–16. [PubMed: 23220263]
71. Pan Y. Mitochondria, reactive oxygen species, and chronological aging: a message from yeast. Exp Gerontol. 2011; 46:847–852. [PubMed: 21884780]
72. Pan Y, Schroeder EA, Ocampo A, Barrientos A, Shadel GS. Regulation of yeast chronological life span by TORC1 via adaptive mitochondrial ROS signaling. Cell Metab. 2011; 13:668–678. [PubMed: 21641548]
73. Li B, Skinner C, Castello PR, Kato M, Easlon E, Xie L, Li T, Lu SP, Wang C, Tsang F, Poyton RO, Lin SJ. Identification of potential calorie restriction-mimicking yeast mutants with increased mitochondrial respiratory chain and nitric oxide levels. J Aging Res. 2011; 2011:673185. [PubMed: 21584246]
74. Mesquita A, Weinberger M, Silva A, Sampaio-Marques B, Almeida B, Leao C, Costa V, Rodrigues F, Burhans WC, Ludovico P. Caloric restriction or catalase inactivation extends yeast chronological lifespan by inducing H2O2 and superoxide dismutase activity. Proc Natl Acad Sci U S A. 2010; 107:15123–15128. [PubMed: 20696905]
75. Lu SP, Lin SJ. Regulation of yeast sirtuins by NAD(+) metabolism and calorie restriction. Biochim Biophys Acta. 2010; 1804:1567–1575. [PubMed: 19818879]
76. Imai S, Guarente L. Ten years of NAD-dependent SIR2 family deacetylases: implications for metabolic diseases. Trends Pharmacol Sci. 2010; 31:212–220. [PubMed: 20226541]
77. Erjavec N, Nystrom T. Sir2p-dependent protein segregation gives rise to a superior reactive oxygen species management in the progeny of Saccharomyces cerevisiae. Proc Natl Acad Sci U S A. 2007; 104:10877–10881. [PubMed: 17581878]

78. Erjavec N, Larsson L, Grantham J, Nystrom T. Accelerated aging and failure to segregate damaged proteins in Sir2 mutants can be suppressed by overproducing the protein aggregation-remodeling factor Hsp104p. Genes Dev. 2007; 21:2410–2421. [PubMed: 17908928]

79. Weinberger M, Feng L, Paul A, Smith DL Jr, Hontz RD, Smith JS, Vujcic M, Singh KK, Huberman JA, Burhans WC. DNA replication stress is a determinant of chronological lifespan in budding yeast. PLoS One. 2007; 2:e748. [PubMed: 17710147]

80. Andersen MP, Nelson ZW, Hetrick ED, Gottschling DE. A genetic screen for increased loss of heterozygosity in Saccharomyces cerevisiae. Genetics. 2008; 179:1179–1195. [PubMed: 18562670]

81. Unal E, Kinde B, Amon A. Gametogenesis eliminates age-induced cellular damage and resets life span in yeast. Science. 2011; 332:1554–1557. [PubMed: 21700873]

82. Anderson RM, Bitterman KJ, Wood JG, Medvedik O, Cohen H, Lin SS, Manchester JK, Gordon JI, Sinclair DA. Manipulation of a nuclear NAD+ salvage pathway delays aging without altering steady-state NAD+ levels. J Biol Chem. 2002; 277:18881–18890. [PubMed: 11884393]

83. Matecic M, Smith DL, Pan X, Maqani N, Bekiranov S, Boeke JD, Smith JS. A microarray-based genetic screen for yeast chronological aging factors. PLoS Genet. 2010; 6:e1000921. [PubMed: 20421943]

84. Hughes AL, Gottschling DE. An early age increase in vacuolar pH limits mitochondrial function and lifespan in yeast. Nature. 2012; 492:261–265. [PubMed: 23172144]

85. Fabrizio P, Hoon S, Shamalnasab M, Galbani A, Wei M, Giaever G, Nislow C, Longo VD. Genome-wide screen in Saccharomyces cerevisiae identifies vacuolar protein sorting, autophagy, biosynthetic, and tRNA methylation genes involved in life span regulation. PLoS Genet. 2010; 6:e1001024. [PubMed: 20657825]

86. Steffen KK, McCormick MA, Pham KM, MacKay VL, Delaney JR, Murakami CJ, Kaeberlein M, Kennedy BK. Ribosome deficiency protects against ER stress in Saccharomyces cerevisiae. Genetics. 2012; 191:107–118. [PubMed: 22377630]

87. Bilinski T, Bartosz G. Hypothesis: cell volume limits cell divisions. Acta Biochim Pol. 2006; 53:833–835. [PubMed: 17106512]

88. Yang J, Dungrawala H, Hua H, Manukyan A, Abraham L, Lane W, Mead H, Wright J, Schneider BL. Cell size and growth rate are major determinants of replicative lifespan. Cell Cycle. 2011; 10:144–155. [PubMed: 21248481]

89. Bilinski T, Zadrag-Tecza R, Bartosz G. Hypertrophy hypothesis as an alternative explanation of the phenomenon of replicative aging of yeast. FEMS Yeast Res. 2012; 12:97–101. [PubMed: 22093953]

90. Delaney JR, Ahmed U, Chou A, Sim S, Carr D, Murakami CJ, Schleit J, Sutphin GL, An EH, Castanza A, Fletcher M, Higgins S, Jelic M, Klum S, Muller B, Peng ZJ, Rai D, Ros V, Singh M, Wende HV, Kennedy BK, Kaeberlein M. Stress profiling of longevity mutants identifies Afg3 as a mitochondrial determinant of cytoplasmic mRNA translation and aging. Aging Cell. 2013; 12:156–166. [PubMed: 23167605]

91. Schleit J, Johnson SC, Bennett CF, Simko M, Trongtham N, Castanza A, Hsieh EJ, Moller RM, Wasko BM, Delaney JR, Sutphin GL, Carr D, Murakami CJ, Tocchi A, Xian B, Chen W, Yu T, Goswami S, Higgins S, Jeong KS, Kim JR, Klum S, Liao E, Lin MS, Lo W, Miller H, Olsen B, Peng ZJ, Pollard T, Pradeep P, Pruett D, Rai D, Ros V, Singh M, Spector BL, Wende HV, An EH, Fletcher M, Jelic M, Rabinovitch PS, Maccoss MJ, Han JD, Kennedy BK, Kaeberlein M. Molecular mechanisms underlying genotype-dependent responses to dietary restriction. Aging Cell. 2013

92. Kruegel U, Robison B, Dange T, Kahlert G, Delaney JR, Kotireddy S, Tsuchiya M, Tsuchiyama S, Murakami CJ, Schleit J, Sutphin G, Carr D, Tar K, Dittmar G, Kaeberlein M, Kennedy BK, Schmidt M. Elevated proteasome capacity extends replicative lifespan in Saccharomyces cerevisiae. PLoS Genet. 2011; 7:e1002253. [PubMed: 21931558]

93. Burtner CR, Murakami CJ, Kennedy BK, Kaeberlein M. A molecular mechanism of chronological aging in yeast. Cell Cycle. 2009; 8:1256–1270. [PubMed: 19305133]

94. Murakami C, Delaney JR, Chou A, Carr D, Schleit J, Sutphin GL, An EH, Castanza AS, Fletcher M, Goswami S, Higgins S, Holmberg M, Hui J, Jelic M, Jeong KS, Kim JR, Klum S, Liao E, Lin

MS, Lo W, Miller H, Moller R, Peng ZJ, Pollard T, Pradeep P, Pruett D, Rai D, Ros V, Schuster A, Singh M, Spector BL, Vander Wende H, Wang AM, Wasko BM, Olsen B, Kaeberlein M. pH neutralization protects against reduction in replicative lifespan following chronological aging in yeast. Cell Cycle. 2012; 11:3087–3096. [PubMed: 22871733]

95. Murakami CJ, Wall V, Basisty N, Kaeberlein M. Composition and acidification of the culture medium influences chronological aging similarly in vineyard and laboratory yeast. PLoS One. 2011; 6:e24530. [PubMed: 21949725]
96. Longo VD, Shadel GS, Kaeberlein M, Kennedy B. Replicative and chronological aging in Saccharomyces cerevisiae. Cell Metab. 2012; 16:18–31. [PubMed: 22768836]
97. Wu Z, Liu SQ, Huang D. Dietary restriction depends on nutrient composition to extend chronological lifespan in budding yeast Saccharomyces cerevisiae. PLoS One. 2013; 8:e64448. [PubMed: 23691220]
98. Fabrizio P, Gattazzo C, Battistella L, Wei M, Cheng C, McGrew K, Longo VD. Sir2 blocks extreme life-span extension. Cell. 2005; 123:655–667. [PubMed: 16286010]
99. Kaeberlein M, Andalis AA, Fink GR, Guarente L. High osmolarity extends life span in Saccharomyces cerevisiae by a mechanism related to calorie restriction. Mol Cell Biol. 2002; 22:8056–8066. [PubMed: 12391171]
100. Easlon E, Tsang F, Dilova I, Wang C, Lu SP, Skinner C, Lin SJ. The dihydrolipoamide acetyltransferase is a novel metabolic longevity factor and is required for calorie restriction-mediated life span extension. J Biol Chem. 2007; 282:6161–6171. [PubMed: 17200108]
101. Easlon E, Tsang F, Skinner C, Wang C, Lin SJ. The malate-aspartate NADH shuttle components are novel metabolic longevity regulators required for calorie restriction-mediated life span extension in yeast. Genes Dev. 2008; 22:931–944. [PubMed: 18381895]
102. Smith DL Jr, McClure JM, Matecic M, Smith JS. Calorie restriction extends the chronological lifespan of Saccharomyces cerevisiae independently of the Sirtuins. Aging Cell. 2007; 6:649–662. [PubMed: 17711561]
103. Wei M, Fabrizio P, Hu J, Ge H, Cheng C, Li L, Longo VD. Life span extension by calorie restriction depends on Rim15 and transcription factors downstream of Ras/PKA, Tor, and Sch9. PLoS Genet. 2008; 4:e13. [PubMed: 18225956]
104. Kaeberlein M, Kirkland KT, Fields S, Kennedy BK. Sir2-independent life span extension by calorie restriction in yeast. PLoS Biol. 2004; 2:1381–1387.
105. Lin YY, Lu JY, Zhang J, Walter W, Dang W, Wan J, Tao SC, Qian J, Zhao Y, Boeke JD, Berger SL, Zhu H. Protein acetylation microarray reveals that NuA4 controls key metabolic target regulating gluconeogenesis. Cell. 2009; 136:1073–1084. [PubMed: 19303850]
106. Hachinohe M, Yamane M, Akazawa D, Ohsawa K, Ohno M, Terashita Y, Masumoto H. A reduction in age-enhanced gluconeogenesis extends lifespan. PLoS One. 2013; 8:e54011. [PubMed: 23342062]
107. Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P. Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. Nature. 2005; 434:113–118. [PubMed: 15744310]
108. Fabrizio P, Pozza F, Pletcher SD, Gendron CM, Longo VD. Regulation of longevity and stress resistance by Sch9 in yeast. Science (New York, N Y. 2001; 292:288–290.
109. Kaeberlein M, Powers RW 3rd, Steffen KK, Westman EA, Hu D, Dang N, Kerr EO, Kirkland KT, Fields S, Kennedy BK. Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. Science (New York, N Y. 2005; 310:1193–1196.
110. Lin SJ, Kaeberlein M, Andalis AA, Sturtz LA, Defossez PA, Culotta VC, Fink GR, Guarente L. Calorie restriction extends Saccharomyces cerevisiae lifespan by increasing respiration. Nature. 2002; 418:344–348. [PubMed: 12124627]
111. Bitterman KJ, Anderson RM, Cohen HY, Latorre-Esteves M, Sinclair DA. Inhibition of silencing and accelerated aging by nicotinamide, a putative negative regulator of yeast sir2 and human SIRT1. J Biol Chem. 2002; 277:45099–45107. [PubMed: 12297502]
112. Sauve AA, Schramm VL. Sir2 regulation by nicotinamide results from switching between base exchange and deacetylation chemistry. Biochemistry. 2003; 42:9249–9256. [PubMed: 12899610]

113. Schmidt MT, Smith BC, Jackson MD, Denu JM. Coenzyme specificity of Sir2 protein deacetylases: implications for physiological regulation. J Biol Chem. 2004; 279:40122–40129. [PubMed: 15269219]
114. Gallo CM, Smith DL Jr, Smith JS. Nicotinamide clearance by Pnc1 directly regulates Sir2-mediated silencing and longevity. Mol Cell Biol. 2004; 24:1301–1312. [PubMed: 14729974]
115. Lin SJ, Ford E, Haigis M, Liszt G, Guarente L. Calorie restriction extends yeast life span by lowering the level of NADH. Genes Dev. 2004; 18:12–16. [PubMed: 14724176]
116. Tanny JC, Kirkpatrick DS, Gerber SA, Gygi SP, Moazed D. Budding yeast silencing complexes and regulation of Sir2 activity by protein-protein interactions. Mol Cell Biol. 2004; 24:6931–6946. [PubMed: 15282295]
117. Bedalov A, Hirao M, Posakony J, Nelson M, Simon JA. NAD+-dependent deacetylase Hst1p controls biosynthesis and cellular NAD+ levels in Saccharomyces cerevisiae. Mol Cell Biol. 2003; 23:7044–7054. [PubMed: 12972620]
118. Li M, Valsakumar V, Poorey K, Bekiranov S, Smith JS. Genome-wide analysis of functional sirtuin chromatin targets in yeast. Genome biology. 2013; 14:R48. [PubMed: 23710766]
119. Auesukaree C, Homma T, Tochio H, Shirakawa M, Kaneko Y, Harashima S. Intracellular phosphate serves as a signal for the regulation of the PHO pathway in Saccharomyces cerevisiae. J Biol Chem. 2004; 279:17289–17294. [PubMed: 14966138]
120. Lenburg ME, O'Shea EK. Signaling phosphate starvation. Trends Biochem Sci. 1996; 21:383–387. [PubMed: 8918192]
121. Mouillon JM, Persson BL. New aspects on phosphate sensing and signalling in Saccharomyces cerevisiae. FEMS Yeast Res. 2006; 6:171–176. [PubMed: 16487340]
122. Xu YF, Letisse F, Absalan F, Lu W, Kuznetsova E, Brown G, Caudy AA, Yakunin AF, Broach JR, Rabinowitz JD. Nucleotide degradation and ribose salvage in yeast. Mol Syst Biol. 2013; 9:665. [PubMed: 23670538]
123. Bogan KL, Brenner C. Nicotinic acid, nicotinamide, and nicotinamide riboside: a molecular evaluation of NAD+ precursor vitamins in human nutrition. Annu Rev Nutr. 2008; 28:115–130. [PubMed: 18429699]
124. Sasaki Y, Araki T, Milbrandt J. Stimulation of nicotinamide adenine dinucleotide biosynthetic pathways delays axonal degeneration after axotomy. J Neurosci. 2006; 26:8484–8491. [PubMed: 16914673]
125. Ramsey KM, Mills KF, Satoh A, Imai S. Age-associated loss of Sirt1-mediated enhancement of glucose-stimulated insulin secretion in beta cell-specific Sirt1-overexpressing (BESTO) mice. Aging Cell. 2008; 7:78–88. [PubMed: 18005249]
126. Nikiforov A, Dolle C, Niere M, Ziegler M. Pathways and subcellular compartmentation of NAD biosynthesis in human cells: from entry of extracellular precursors to mitochondrial NAD generation. J Biol Chem. 2011; 286:21767–21778. [PubMed: 21504897]
127. Garavaglia S, Bruzzone S, Cassani C, Canella L, Allegrone G, Sturla L, Mannino E, Millo E, De Flora A, Rizzi M. The high-resolution crystal structure of periplasmic Haemophilus influenzae NAD nucleotidase reveals a novel enzymatic function of human CD73 related to NAD metabolism. Biochem J. 2012; 441:131–141. [PubMed: 21933152]
128. Schmidt-Brauns J, Herbert M, Kemmer G, Kraiss A, Schlor S, Reidl J. Is a NAD pyrophosphatase activity necessary for Haemophilus influenzae type b multiplication in the blood stream? Int J Med Microbiol. 2001; 291:219–225. [PubMed: 11554562]
129. Giots F, Donaton MC, Thevelein JM. Inorganic phosphate is sensed by specific phosphate carriers and acts in concert with glucose as a nutrient signal for activation of the protein kinase A pathway in the yeast Saccharomyces cerevisiae. Mol Microbiol. 2003; 47:1163–1181. [PubMed: 12581367]
130. Mouillon JM, Persson BL. Inhibition of the protein kinase A alters the degradation of the high-affinity phosphate transporter Pho84 in Saccharomyces cerevisiae. Curr Genet. 2005; 48:226–234. [PubMed: 16160831]
131. Lundh F, Mouillon JM, Samyn D, Stadler K, Popova Y, Lagerstedt JO, Thevelein JM, Persson BL. Molecular mechanisms controlling phosphate-induced downregulation of the yeast Pho84 phosphate transporter. Biochemistry. 2009; 48:4497–4505. [PubMed: 19348508]

132. Popova Y, Thayumanavan P, Lonati E, Agrochao M, Thevelein JM. Transport and signaling through the phosphate-binding site of the yeast Pho84 phosphate transceptor. Proc Natl Acad Sci U S A. 2010; 107:2890–2895. [PubMed: 20133652]

133. Samyn DR, Ruiz-Pavon L, Andersson MR, Popova Y, Thevelein JM, Persson BL. Mutational analysis of putative phosphate- and proton-binding sites in the Saccharomyces cerevisiae Pho84 phosphate:H(+) transceptor and its effect on signalling to the PKA and PHO pathways. Biochem J. 2012; 445:413–422. [PubMed: 22587366]

134. Kang HJ, Chang M, Kang CM, Park YS, Yoon BJ, Kim TH, Yun CW. The expression of PHO92 is regulated by Gcr1, and Pho92 is involved in glucose metabolism in Saccharomyces cerevisiae. Curr Genet. 2014

135. Kang HJ, Jeong SJ, Kim KN, Baek IJ, Chang M, Kang CM, Park YS, Yun CW. A novel protein, Pho92, has a conserved YTH domain and regulates phosphate metabolism by decreasing the mRNA stability of PHO4 in Saccharomyces cerevisiae. Biochem J. 2014; 457:391–400. [PubMed: 24206186]

136. Carroll AS, Bishop AC, DeRisi JL, Shokat KM, O'Shea EK. Chemical inhibition of the Pho85 cyclin-dependent kinase reveals a role in the environmental stress response. Proc Natl Acad Sci U S A. 2001; 98:12578–12583. [PubMed: 11675494]

137. Swinnen E, Wanke V, Roosen J, Smets B, Dubouloz F, Pedruzzi I, Cameroni E, De Virgilio C, Winderickx J. Rim15 and the crossroads of nutrient signalling pathways in Saccharomyces cerevisiae. Cell Div. 2006; 1:3. [PubMed: 16759348]

138. Roosen J, Engelen K, Marchal K, Mathys J, Griffioen G, Cameroni E, Thevelein JM, De Virgilio C, De Moor B, Winderickx J. PKA and Sch9 control a molecular switch important for the proper adaptation to nutrient availability. Mol Microbiol. 2005; 55:862–880. [PubMed: 15661010]

139. Medvedik O, Lamming DW, Kim KD, Sinclair DA. MSN2 and MSN4 link calorie restriction and TOR to sirtuin-mediated lifespan extension in Saccharomyces cerevisiae. PLoS Biol. 2007; 5:e261. [PubMed: 17914901]

140. Anderson RM, Bitterman KJ, Wood JG, Medvedik O, Sinclair DA. Nicotinamide and PNC1 govern lifespan extension by calorie restriction in Saccharomyces cerevisiae. Nature. 2003; 423:181–185. [PubMed: 12736687]

141. Silva RM, Duarte IC, Paredes JA, Lima-Costa T, Perrot M, Boucherie H, Goodfellow BJ, Gomes AC, Mateus DD, Moura GR, Santos MA. The yeast PNC1 longevity gene is up-regulated by mRNA mistranslation. PLoS One. 2009; 4:e5212. [PubMed: 19381334]

142. Shama S, Lai CY, Antoniazzi JM, Jiang JC, Jazwinski SM. Heat stress-induced life span extension in yeast. Exp Cell Res. 1998; 245:379–388. [PubMed: 9851879]

143. Lamming DW, Wood JG, Sinclair DA. Small molecules that regulate lifespan: evidence for xenohormesis. Mol Microbiol. 2004; 53:1003–1009. [PubMed: 15306006]

144. Jia SH, Li Y, Parodo J, Kapus A, Fan L, Rotstein OD, Marshall JC. Pre-B cell colony-enhancing factor inhibits neutrophil apoptosis in experimental inflammation and clinical sepsis. J Clin Invest. 2004; 113:1318–1327. [PubMed: 15124023]

145. van der Veer E, Nong Z, O'Neil C, Urquhart B, Freeman D, Pickering JG. Pre-B-cell colony-enhancing factor regulates NAD+-dependent protein deacetylase activity and promotes vascular smooth muscle cell maturation. Circ Res. 2005; 97:25–34. [PubMed: 15947248]

146. Schmeisser K, Mansfeld J, Kuhlow D, Weimer S, Priebe S, Heiland I, Birringer M, Groth M, Segref A, Kanfi Y, Price NL, Schmeisser S, Schuster S, Pfeiffer AF, Guthke R, Platzer M, Hoppe T, Cohen HY, Zarse K, Sinclair DA, Ristow M. Role of sirtuins in lifespan regulation is linked to methylation of nicotinamide. Nat Chem Biol. 2013; 9:693–700. [PubMed: 24077178]

147. Grose JH, Bergthorsson U, Roth JR. Regulation of NAD synthesis by the trifunctional NadR protein of Salmonella enterica. J Bacteriol. 2005; 187:2774–2782. [PubMed: 15805524]

148. Ocampo A, Liu J, Barrientos A. NAD+ salvage pathway proteins suppress proteotoxicity in yeast models of neurodegeneration by promoting the clearance of misfolded/oligomerized proteins. Hum Mol Genet. 2013; 22:1699–1708. [PubMed: 23335597]

149. Todisco S, Agrimi G, Castegna A, Palmieri F. Identification of the mitochondrial NAD+ transporter in Saccharomyces cerevisiae. J Biol Chem. 2006; 281:1524–1531. [PubMed: 16291748]

150. Bakker BM, Overkamp KM, van Maris AJ, Kotter P, Luttik MA, van Dijken JP, Pronk JT. Stoichiometry and compartmentation of NADH metabolism in Saccharomyces cerevisiae. FEMS Microbiol Rev. 2001; 25:15–37. [PubMed: 11152939]

151. Houtkooper RH, Canto C, Wanders RJ, Auwerx J. The secret life of NAD+: an old metabolite controlling new metabolic signaling pathways. Endocr Rev. 2010; 31:194–223. [PubMed: 20007326]

152. Chen D, Bruno J, Easlon E, Lin SJ, Cheng HL, Alt FW, Guarente L. Tissue-specific regulation of SIRT1 by calorie restriction. Genes Dev. 2008; 22:1753–1757. [PubMed: 18550784]

153. Bordone L, Motta MC, Picard F, Robinson A, Jhala US, Apfeld J, McDonagh T, Lemieux M, McBurney M, Szilvasi A, Easlon EJ, Lin SJ, Guarente L. Sirt1 regulates insulin secretion by repressing UCP2 in pancreatic beta cells. PLoS Biol. 2006; 4:e31. [PubMed: 16366736]

154. Eto K, Tsubamoto Y, Terauchi Y, Sugiyama T, Kishimoto T, Takahashi N, Yamauchi N, Kubota N, Murayama S, Aizawa T, Akanuma Y, Aizawa S, Kasai H, Yazaki Y, Kadowaki T. Role of NADH shuttle system in glucose-induced activation of mitochondrial metabolism and insulin secretion. Science (New York, N Y. 1999; 283:981–985.

155. Haigis MC, Mostoslavsky R, Haigis KM, Fahie K, Christodoulou DC, Murphy AJ, Valenzuela DM, Yancopoulos GD, Karow M, Blander G, Wolberger C, Prolla TA, Weindruch R, Alt FW, Guarente L. SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic beta cells. Cell. 2006; 126:941–954. [PubMed: 16959573]

156. Noda T, Klionsky DJ. The quantitative Pho8Delta60 assay of nonspecific autophagy. Methods Enzymol. 2008; 451:33–42. [PubMed: 19185711]

157. Vickers MF, Yao SY, Baldwin SA, Young JD, Cass CE. Nucleoside transporter proteins of Saccharomyces cerevisiae. Demonstration of a transporter (FUI1) with high uridine selectivity in plasma membranes and a transporter (FUN26) with broad nucleoside selectivity in intracellular membranes. J Biol Chem. 2000; 275:25931–25938. [PubMed: 10827169]

158. Wiederhold E, Gandhi T, Permentier HP, Breitling R, Poolman B, Slotboom DJ. The yeast vacuolar membrane proteome. Mol Cell Proteomics. 2009; 8:380–392. [PubMed: 19001347]

159. Young JD, Yao SY, Sun L, Cass CE, Baldwin SA. Human equilibrative nucleoside transporter (ENT) family of nucleoside and nucleobase transporter proteins. Xenobiotica. 2008; 38:995–1021. [PubMed: 18668437]

160. Baldwin SA, Yao SY, Hyde RJ, Ng AM, Foppolo S, Barnes K, Ritzel MW, Cass CE, Young JD. Functional characterization of novel human and mouse equilibrative nucleoside transporters (hENT3 and mENT3) located in intracellular membranes. J Biol Chem. 2005; 280:15880–15887. [PubMed: 15701636]

161. Endo Y, Obata T, Murata D, Ito M, Sakamoto K, Fukushima M, Yamasaki Y, Yamada Y, Natsume N, Sasaki T. Cellular localization and functional characterization of the equilibrative nucleoside transporters of antitumor nucleosides. Cancer Sci. 2007; 98:1633–1637. [PubMed: 17711502]

162. Kato M, Lin SJ. YCL047C/POF1 Is a Novel Nicotinamide Mononucleotide Adenylyltransferase (NMNAT) in Saccharomyces cerevisiae. J Biol Chem. 2014; 289:15577–15587. [PubMed: 24759102]

163. AbdelRaheim SR, Cartwright JL, Gasmi L, McLennan AG. The NADH diphosphatase encoded by the Saccharomyces cerevisiae NPY1 nudix hydrolase gene is located in peroxisomes. Arch Biochem Biophys. 2001; 388:18–24. [PubMed: 11361135]

164. Clapper DL, Walseth TF, Dargie PJ, Lee HC. Pyridine nucleotide metabolites stimulate calcium release from sea urchin egg microsomes desensitized to inositol trisphosphate. J Biol Chem. 1987; 262:9561–9568. [PubMed: 3496336]

165. Guse AH, Lee HC. NAADP: a universal Ca2+ trigger. Sci Signal. 2008; 1:re10. [PubMed: 18984909]

166. Cheng W, Roth J. Isolation of NAD cycle mutants defective in nicotinamide mononucleotide deamidase in Salmonella typhimurium. J Bacteriol. 1995; 177:6711–6717. [PubMed: 7592458]

167. Revollo JR, Grimm AA, Imai S. The NAD biosynthesis pathway mediated by nicotinamide phosphoribosyltransferase regulates Sir2 activity in mammalian cells. J Biol Chem. 2004; 279:50754–50763. [PubMed: 15381699]

Highlights

- Pyridine nucleotides and their metabolites play important roles in variety of cellular processes
- Crosstalk between sirtuins, NAD^+- and nutrient-sensing pathways renders metabolic flexibility
- Compartmentalization helps regulate the homeostasis of pyridine nucleotides

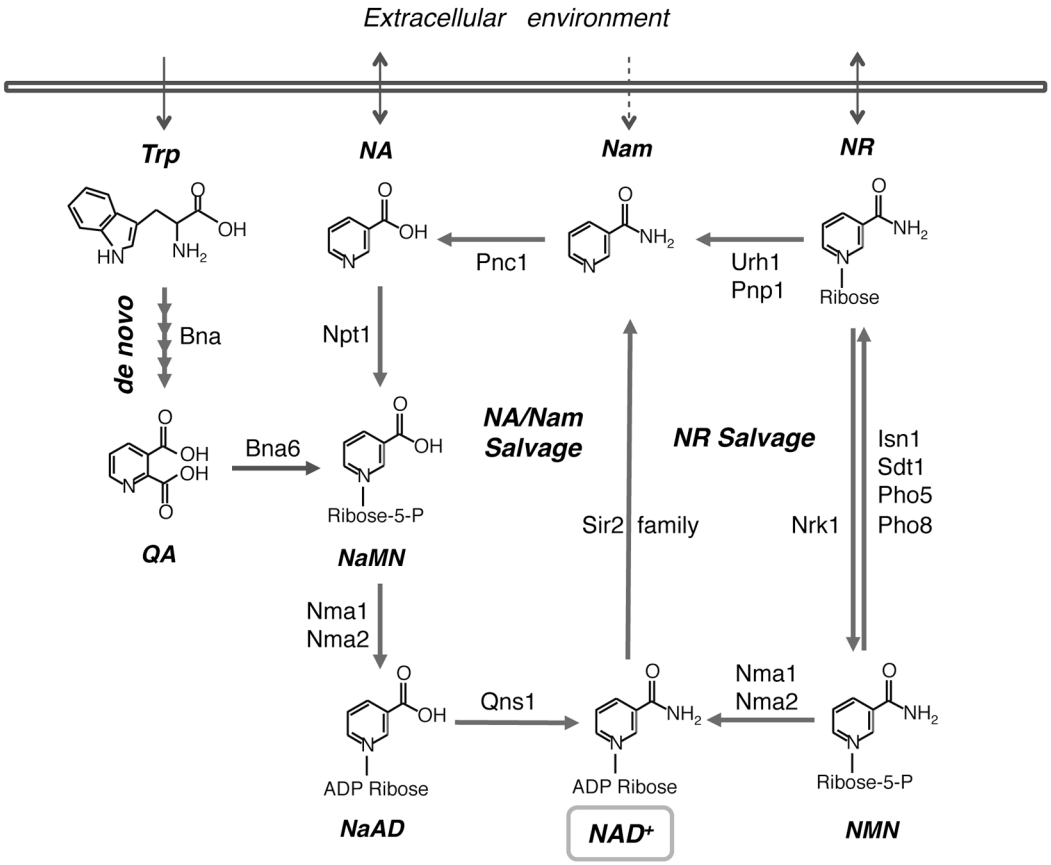

Figure 1.

A simplified model of the *Saccharomyces cerevisiae* NAD^+ synthesis pathway. In yeast, NAD^+ is synthesized *de novo* from tryptophan (*Trp*) and by salvaging nicotinic acid (*NA*), nicotinamide (*Nam*), quinolinic acid (*QA*) and nicotinamide riboside (*NR*). Cells can also salvage nicotinic acid riboside (*NaR*) by converting it to *NA* or *NaMN* (nicotinic acid mononucleotide). For simplicity, *NaR* salvaging is not shown in this figure. *NaAD*, deamido *NAD*$^+$. *NMN*, nicotinamide mononucleotide. NAD^+ and NAD^+ intermediates are italicized. Abbreviations of protein names catalyzing each step are shown in bold.

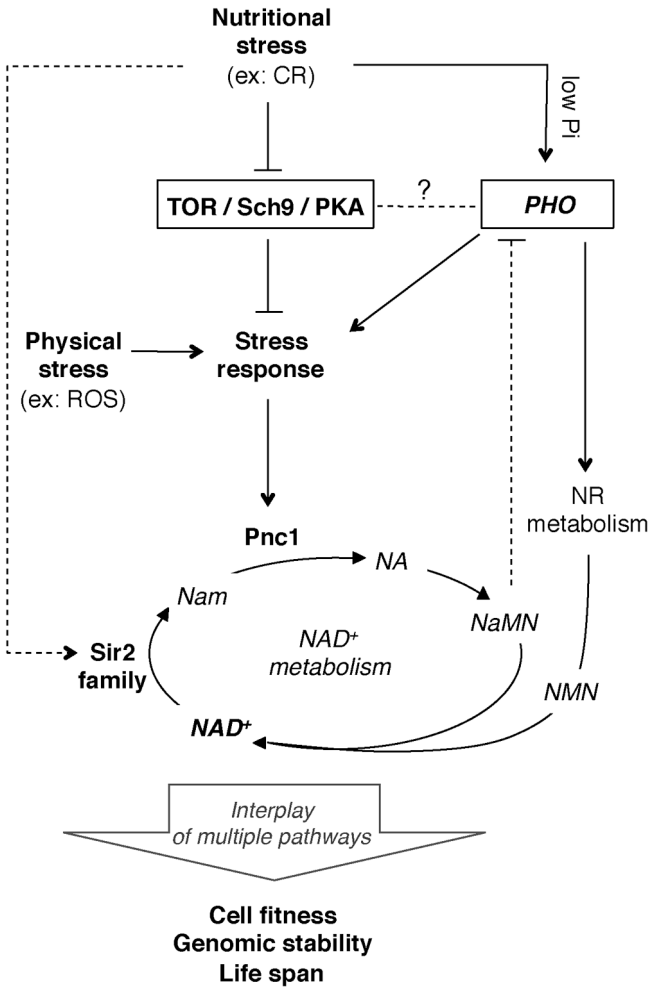

Figure 2.

Interactions of NAD^+ metabolism and multiple cellular signaling pathways contribute to the regulation of cell function. NAD^+ and NAD^+ intermediates are italicized. Abbreviations of protein names catalyzing specific steps are shown in bold. Dashed lines indicate that additional evidence is required to reveal the molecular mechanisms.

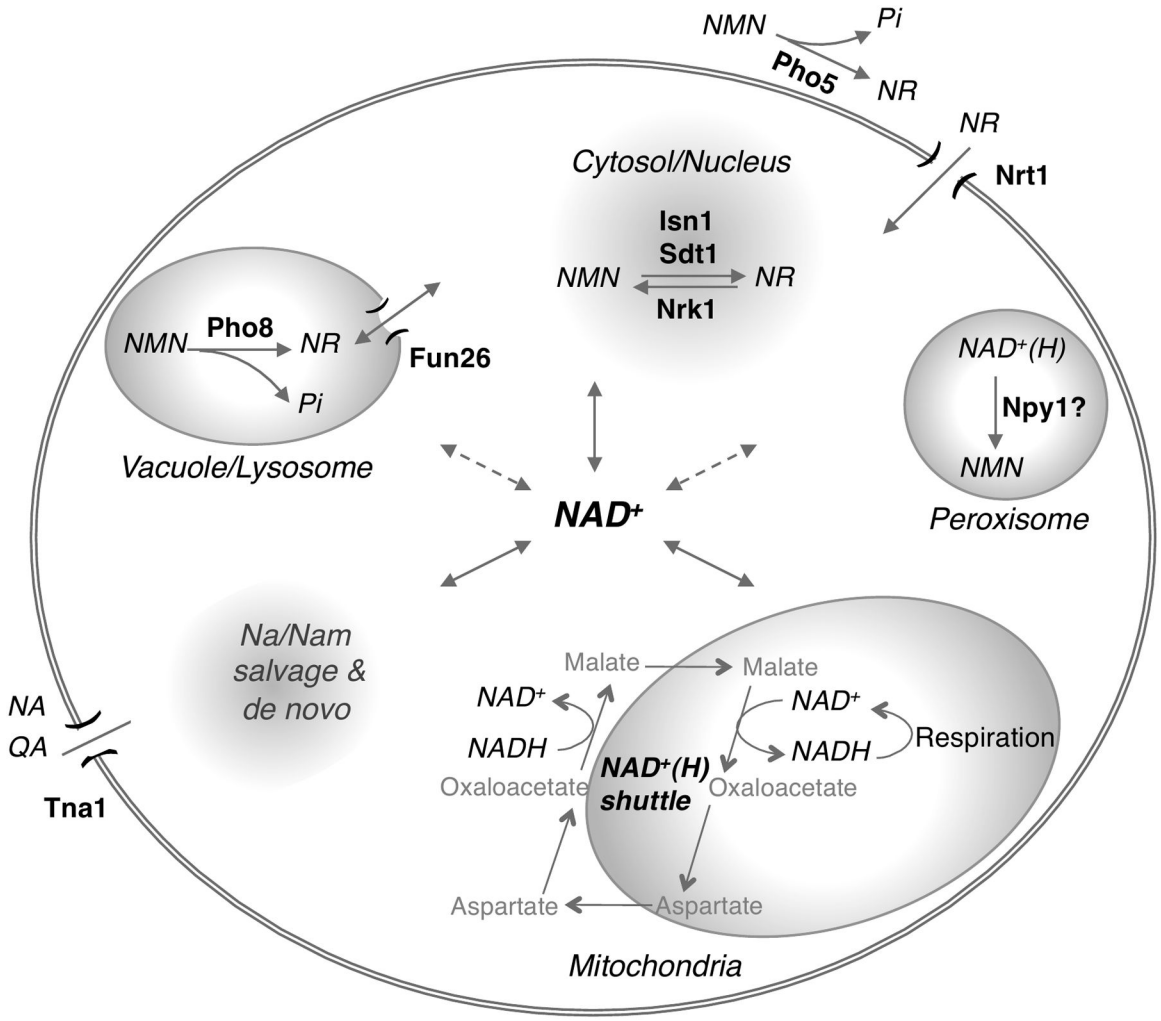

Figure 3.

Compartmentalization of intracellular NAD^+ and its derivates regulates NAD^+ homeostasis. Intracellular compartments contribute to the storage and supply of NAD^+ intermediates such as NR and NMN. Vacuole is a plausible site for NR storage supported by the discovery of a putative NR transporter Fun26 and a NR producing nucleotidase Pho8. Periplasmic nucelotidase Pho5 and plasma membrane localized NR transporter Nrt1 facilitate the utilization of extracellular NMN and NR pools, respectively. The NADH shuttle systems do not directly affect NAD^+ metabolism, instead, they function to balance redox equivalents between the mitochondrial and the cytosolic/nuclear pools to regulate the NAD^+/NADH ratio. The malate-aspartate shuttle is shown here as an example. Abbreviations of protein names catalyzing specific steps are shown in bold. Dashed lines indicate that additional evidence is required to reveal the molecular mechanisms.