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REVIEW

Less is more: Nutrient limitation induces cross-talk of nutrient sensing pathways with NAD^+ homeostasis and contributes to longevity

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Abstract **Nutrient sensing pathways and their regulation grant cells control over their metabolism and growth in response to changing nutrients. Factors that regulate nutrient sensing can also modulate longevity. Reduced activity of nutrient sensing pathways such as glucose-sensing PKA, nitrogen-sensing TOR and S6 kinase homolog Sch9 have been linked to increased life span in the yeast, *Saccharomyces cerevisiae*, and higher eukaryotes. Recently, reduced activity of amino acid sensing SPS pathway was also shown to increase yeast life span. Life span extension by reduced SPS activity requires enhanced NAD^+ (nicotinamide adenine dinucleotide, oxidized form) and nicotinamide riboside (NR, a NAD^+ precursor) homeostasis. Maintaining adequate NAD^+ pools has been shown to play key roles in life span extension, but factors regulating NAD^+ metabolism and homeostasis are not completely understood. Recently, NAD^+ metabolism was also linked to the phosphate (Pi)-sensing *PHO* pathway in yeast. Canonical *PHO* activation requires Pi-starvation. Interestingly, NAD^+ depletion without Pi-starvation was sufficient to induce *PHO* activation, increasing NR production and mobilization. Moreover, SPS signaling appears to function in parallel with *PHO* signaling components to regulate NR/NAD^+ homeostasis. These studies suggest that NAD^+ metabolism is likely controlled by and/or coordinated with multiple nutrient sensing pathways. Indeed, cross-regulation of *PHO*, PKA, TOR and Sch9 pathways was reported to potentially affect NAD^+ metabolism; though detailed mechanisms remain unclear. This review discusses yeast longevity-related nutrient sensing pathways and possible mechanisms of life span extension, regulation of NAD^+ homeostasis, and cross-talk among nutrient sensing pathways and NAD^+ homeostasis.**

Keywords nutrient sensing, NAD^+ homeostasis, yeast longevity

Introduction

Nutrient sensing pathways are integral to growth and survival of all living organisms. A multitude of nutrient sensing mechanisms allow organisms to regulate cell activity with fine control. To this end, many sensor pathways cross-regulate each other or cross-talk (Fig. 1). Much research has been done in the budding yeast, *Saccharomyces cerevisiae*, on sensing of nutrients such as amino acids, nitrogen, glucose and phosphate.

Reduced activity of nutrient sensing pathways such as glucose-sensing cyclic AMP-protein kinase A (PKA), nitrogen-sensing target of rapamycin (TOR) and the S6 kinase homolog Sch9 have been linked to increased life span in yeast and higher eukaryotes (Lin et al., 2000; Fabrizio et al., 2001; Kaeberlein et al., 2005b; Wei et al., 2008; Wang et al., 2009; Longo and Fabrizio, 2012). Recent studies have proposed several molecular mechanisms for yeast life span extension (see the section "Factors contributing to life span extension"). Among these, metabolic factors such as NAD^+ play an important role. In this review, we start by discussing longevity related nutrient sensing pathways and proposed mechanisms of life span extension, followed by NAD^+ metabolism and signaling pathways connected to NAD^+ metabolism.

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PKA and carbon-source sensing pathways

Yeast cells can sense and uptake preferred carbon sources

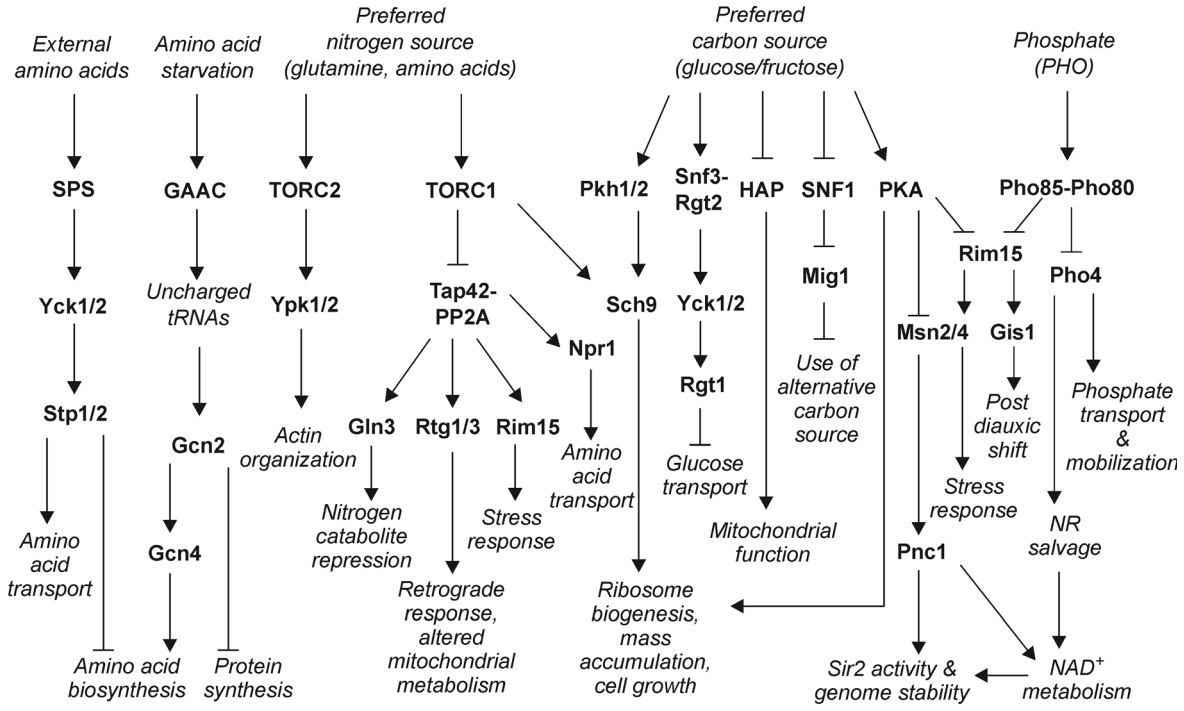

Figure 1 Nutrient sensing pathways that have been linked to life span in yeast. Amino acids, preferred nitrogen sources, carbon sources and phosphate are sensed by yeast cells through various signaling pathways. Some of these pathways cross-talk via shared targets. Major functions of each pathway are summarized in the sections "PKA and carbon-source sensing pathways" to "Phosphate sensing pathway. " Abbreviations of the key players shown here are defined in the order of left to right and top to bottom. SPS: Ssy1-Ptr3-Ssy5 amino acid sensing pathway. Yck1/2: yeast casein kinase 1/2. Stp1/2: SPS pathway transcription factors. GAAC: general amino acid control. Gcn2: general control nonderepressible kinase. Gcn4: general control nonderepressible, leucine-zipper transcription factor. TORC2: target of rapamycin complex 2. Ypk1/2: yeast protein kinase; serine/threonine protein kinase. TORC1: target of rapamycin complex 1. Tap42-PP2A: Tap42: two A phosphatase associated protein; PP2A: protein phosphatase 2A; complex. Npr1: nitrogen permease reactivator; kinase. Gln3: glutamine metabolism; TOR transcription factor. Rtg1/3: retrograde response transcription factors. Rim15: serine/threonine kinase. Pkh1/2: PKB-activating kinase homolog 1/2; serine/threonine kinases. Sch9: S6 kinase homolog. Snf3-Rgt2: plasma membrane sensors; Snf3: sucrose non-fermenting; low glucose sensor, Rgt2; restores glucose transport; high glucose sensor. Rgt1: restores glucose transport; glucose-responsive transcription factor. SNF1: sucrose non-fermenting 1; complex. Mig1: multicopy inhibitor of *GAL* gene expression; transcription repressor. PKA: glucose-sensing cyclic AMP-protein kinase A pathway. Msn2/4: zinc finger STRE (stress response element; AGGGG) binding transcription factors. Pnc1: nicotinamide deamidase. Gis1: zinc finger STRE binding transcription factors. Pho85-Pho80: Pho80: cyclin, Pho85: cyclin dependent kinase; complex. Pho4: *PHO* pathway transcription factor.

through many pathways. Although well studied, the exact mechanism of carbon-source activation in some pathways is still unclear. In this section, we introduce five interacting glucose-sensing pathways, which have been connected to life span: The cyclic AMP-protein kinase A (PKA) (cell growth and stress response), SNF1 (alternative carbon source utilization), HAP (mitochondrial function), Snf3-Rgt2 (glucose sensing and transport) and Sch9 (cell growth).

PKA

In the presence of external glucose, PKA is activated, increasing anabolic and decreasing catabolic activity of the cell. Two glucose-sensing pathways, the G-protein coupled receptor (GPCR) system and the monomeric small GTPase, RAS, protein system act upon PKA (Broach 2012; Conrad et al., 2014). The major activator of PKA, RAS, is activated in the presence of glucose by the cytoplasmic GTP exchange factor (GEF), Cdc25. Cdc25 exchanges a GDP for a GTP on the GTPases, Ras1 and Ras2 (Ras1/2), which are tethered to the plasma membrane via post-translational palmitoylation at their C-termini. Ras1/2 are active in their GTP form (GTP-Ras1/2), and inactive in their GDP form (GDP-Ras1/2). Inactivation requires cytoplasmic GTPase activating proteins (GAPs), Ira1 and Ira2, which terminate RAS signaling by stimulating GTPase activity of GTP-Ras1/2, resulting in inactive GDP-Ras1/2. When activated, GTP-Ras1/2 directly activates plasma membrane associated adenylate cyclase (AC), stimulating AC catalytic activity. Activated AC produces cyclic AMP (cAMP) from ATP and the phosphodiesterases, Pde1 and Pde2, terminate cAMP signaling by converting cAMP into AMP. Once produced, cAMP can bind to Bcy1, the cytoplasmic regulatory subunit of PKA, which disassociates from and thus activates the catalytic subunits of

PKA, Tpk1, Tpk2 and Tpk3 (Tpk1–3). How glucose activates the RAS protein system remains unclear. Activation appears to require glucose uptake/catabolism, hexokinase-mediated phosphorylation (Rolland et al., 2000; Thevelein et al., 2000; Conrad et al., 2014) and possibly cellular pH changes (Broach 2012). Once AC is stimulated by the RAS pathway, the GPCR system can also activate AC (Conrad et al., 2014).

The GPCR system consists of the plasma membrane G-protein coupled receptor, Gpr1, which in the presence of glucose shifts conformation and activates Gpa2, a G protein alpha subunit. Gpa2 associates with the plasma membrane via post-translational palmitoylation and myristoylation (Dohlman and Thorner, 2001; Overton et al., 2005). Gpa2 activates both cAMP-dependent and cAMP-independent pathways of PKA activation. In the cAMP-dependent pathway, Gpa2 activates AC, but only after AC has been activated by GTP-Ras1/2 via the RAS protein system. Activated AC then activates PKA as described above. It is peculiar that Gpa2 only activates AC after AC has been stimulated by RAS proteins, since this indicates that extracellular glucose sensing by the GPCR system requires intracellular glucose catabolism to activate its pathway. However, the GPCR system does have a cAMP-independent pathway via Krh1 and Krh2 (G β mimics), which directly activate PKA by lowering affinity between regulatory subunit, Bcy1, and catalytic subunits, Tpk1-3 (Peeters et al., 2006). Perhaps PKA activation through both RAS protein and GPCR systems is an elegant method by which the cell integrates glucose sensing and transport with the levels of glucose catabolism, requiring availability of glycolytic enzymes and phosphate sources to amplify glucose transport. Reduced PKA pathway activity has been shown to extend life span and may mimic calorie restriction (CR) (Lin et al., 2000; Wang et al., 2009) (see the section "Factors contributing to life span extension").

Activated PKA, as shown in Fig. 1, increases various anabolic pathways including ribosome biogenesis, mass accumulation and cell growth and inhibits certain catabolic responses (Broach, 2012). Also, PKA inhibits Rim15-mediated stress response pathway by phosphorylation of Rim15 (serine/threonine kinase). Phosphorylated Rim15 is anchored to the cytoplasm through its binding to 14-3-3 proteins (Bmh1/2) and kept inactive (Wanke et al., 2005). Active Rim15 upregulates various genes whose expression depends on zinc finger transcription factors, Msn2, Msn4, and Gis1. Msn2/4 activate gene expression by binding to stress response element (STRE, AGGGG) in response to diverse stress conditions including nutrient starvation. Gis1 activates transcription through post diauxic shift (PDS, TT/AAGG-GAT) element upon glucose depletion. Rim15 has been shown to directly phosphorylate and activate Msn2 (Lee et al., 2013), but it remains unclear how Rim 15 activates Gis1 and Msn4. PKA also directly regulates Msn2/4 by controlling their nuclear localization: nuclear localization of Msn2/4 is correlated inversely to cAMP levels and PKA activity. Nuclear localization signal sequences (NLS) of Msn2/4 are direct targets of PKA and phosphorylated NLS prevents nuclear import of Msn2/4 (Görner et al., 1998; Görner et al., 2002). PKA may also play a role in NAD^+ metabolism via Msn2/4 regulated Pnc1 expression. Pnc1 is a nicotinamide deamidase, which converts nicotinamide (Nam) to nicotinic acid (NA), contributing to the Nam/NA salvage branch of NAD^+ metabolism (see the subsection Overview of NAD^+ biosynthesis). Nam is also a product of conserved Sir2 (silent information regulator 2) family proteins, which are NAD^+-dependent deacetylases, whose activity is strongly inhibited by Nam. Pnc1 may thus enhance Sir2 activity both by promoting Nam clearance (removing Sir2 inhibitor) as well as by increasing NAD^+ synthesis (providing substrate for Sir2) (see the subsection "Additional roles of NAD^+ intermediates and biosynthetic enzymes").

Snf3-Rgt2

PKA also phosphorylates and activates Rgt1 (restores glucose transport) (Jouandot et al., 2011), a glucose-responsive transcription factor, which is part of the Snf3-Rgt2 glucose sensing pathway (Fig. 1). This leads to increased expression of hexose transporter (*HXT*) genes and therefore increased glucose transport (Kim et al., 2006; Broach 2012; Ljungdahl and Daignan-Fornier, 2012; Conrad et al., 2014). Activation of Rgt1 by PKA requires the activity of Snf3-Rgt2 glucose-sensing pathway, which is composed of a low glucose sensor, Snf3 (sucrose non-fermenting), and its paralogous high glucose sensor, Rgt2 (restores glucose transport). Both are transporter-like proteins with only sensor function, localized to the plasma membrane. In the absence of glucose, the downstream transcription factor, Rgt1, complexes with the corepressor paralogs, Mth1 and Std1. This repression complex recruits the general corepressor complex, Ssn6-Tup1 (also called Cyc8-Tup1), to the *HXT* promoters and thus represses *HXT* gene expression. When glucose binds to Snf3 and Rgt2 sensors, corepressors Mth1 and Std1 are recruited to the plasma membrane where they are phosphorylated by Yck1 and Yck2 (Yck1/2; yeast casein kinase 1/2 homolog) (Moriya and Johnston, 2004; Pasula et al., 2007). Yck1/2 are palmitoylated plasma membrane-bound serine/threonine protein kinases (Moriya and Johnston, 2004; Pasula et al., 2007). Phosphorylation of Mth1 and Std1 by Yck1/2, leads to their ubiquitination by Grr1, an F-box component of a SCF ubiquitin-ligase complex, and subsequent proteasomal degradation (Flick et al., 2003; Kim et al., 2006; Broach 2012). This frees Rgt1 for phosphorylation by PKA, after which phosphorylated Rgt1 binds to *HXT* promoters and promotes *HXT* gene expression, leading to increased glucose transport. Unlike some glucose sensing pathways, where reduced activity leads to increased life span, deletion of Snf3-Rgt2 pathway components has recently been shown to shorten both normal and CR mediated life span as well as decrease mitochondrial function (Choi et al., 2015).

SNF1

SNF1 (sucrose non-fermenting 1) complex is another glucose sensing pathway that affects *HXT* gene expression (Fig. 1). In yeast, glucose depletion activates the SNF1 (serine/threonine kinase) complex, which plays an essential role in derepressing glucose-repressed genes (Celenza and Carlson, 1986), and is required for growth on low-glucose or non-glucose carbon sources such as sucrose (Celenza and Carlson, 1986). SNF1 is a yeast homolog of the mammalian AMP-activated kinase (AMPK), which is a heterotrimeric complex, including a catalytic α subunit and two regulatory subunits, β and γ. Yeast SNF1 complex includes the Snf1 kinase (α), an activating regulatory subunit Snf4 (γ), and one of the three β subunits (Gal83, Sip1 or Sip2) that determine SNF1 localization (Hardie 2007). In mammalian cells, AMPK responds to energy depletion (increased AMP/ATP ratio) and regulates energy homeostasis (Xiao et al., 2007). Unlike mammalian AMPK, yeast SNF1 is not activated by AMP binding, instead it responds directly to glucose level. In response to glucose depletion, activation of Snf1 results from direct phosphorylation of Thr201 in the activation loop of Snf1 (McCartney and Schmidt, 2001; Rubenstein et al., 2008) by three redundant Snf1-activating kinases (Elm1, Tos3 and Sak1) (Hong et al., 2003; Sutherland et al., 2003). Similar to AMPK, SNF1 is regulated primarily by phosphorylation status of its activation loop, however adenine nucleotide mediated allosteric stimulation also plays a role. It has been shown that ADP can enhance SNF1 (and AMPK) activity by binding to the regulatory γ subunit Snf4. Snf4 (when bound to ADP) exerts its regulation by preventing the access of the Reg1-Glc7 (Reg1: resistance to glucose repression, Glc7: glycogen) phosphatase to the activating phosphate on Snf1 (Thr210), thus prolonging Snf1 activation (Mayer et al., 2011). It was also shown that ADP binding to the active loop of Snf1 (Chandrashekarappa et al., 2013) could trigger a conformational change of SNF1 complex, rendering extended resistance to phosphatase activity. Therefore, SNF1/AMPK utilizes at least two different mechanisms for adenine nucleotides (ADP or AMP) to confer protection: through binding with the active site (as ligands) and through binding with the regulatory γ subunit Snf4.

SNF1 mediates derepression of genes for utilizing alternative (non-glucose) carbon sources by inactivation of the transcription repressors, Mig1/2 (multicopy inhibitor of GAL gene expression) (Hahn and Young, 2011). Phosphorylation of Mig1 by SNF1 and Hxk2 prevent its nuclear localization, and thus Mig1 no longer represses its target genes. SNF1 also activates transcription factors responsible for inducing gluconeogenesis (Cat8, Sip4 and Rsd2) (Roth et al., 2004), stress response (Hsf1 and Msn2) (Sanz 2003; Hahn and Thiele, 2004; De Wever et al., 2005), and fatty acid metabolism (Adr1) (Parua et al., 2010). Furthermore, SNF1 also controls gene expression by stimulating chromatin remodeling. For example during glucose depletion, activated SNF1 phosphorylates histone H3, leading to the recruitment of the SAGA complex and acetylation of histone H3 at the promoters of glucose transporter genes (*HXT2* and *HXT4*) (van Oevelen et al., 2006). Also, AMPK has been shown to affect yeast and worm life span (Schulz et al., 2007). In yeast, increased activity of Snf1 was reported to reduce resistance to nutrient deprivation and shorten life span (Ashrafi et al., 2000; Lin et al., 2003; Lu et al., 2011). It is reported that as cells age, Sip2, a regulatory subunit of SNF1 is less acetylated (controlled by NuA4 acetyltransferase and Rpd3 deacetylase) (Lu et al., 2011). Sip2 acetylation enhances its interaction with Snf1 and inhibits Snf1 activity, leading to decreased phosphorylation of Sch9 by SNF1, and longer life span (see the subsection "Sch9"). In a recent study, SNF1 pathway components were shown to be required for life span extension due to increased proteasome activity (Yao et al., 2015). Also, perturbation of Mig1 levels by either overexpression or reduction in catalytic activity had an adverse effect on proteasome-induced life span extension (Yao et al., 2015).

HAP

HAP (Heme activator protein) refers to the Hap2/3/4/5 CCAAT binding complex (Chodosh et al., 1988; Hahn and Guarente, 1988; McNabb et al., 1995), part of another glucose-regulated pathway (Fig. 1). HaP2/3/5 is a heme-activated, glucose-repressed global transcription factor complex, which is activated during respiratory growth on non-fermentable carbon sources (Zitomer and Lowry, 1992; Gancedo 1998; Schüller 2003). Hap2/3/5 form a constitutively expressed DNA binding complex (Hahn and Guarente, 1988; McNabb et al., 1995; McNabb and Pinto, 2005) with Hap4 as the activation component. Hap4 expression increases upon glucose depletion, and this increase alone induces gene expression of HAP targets (Forsburg and Guarente, 1989; DeRisi et al., 1997; Lascaris et al., 2003). Hap1 does not complex with Hap2/3/4/5 and has a distinct function. Hap1 activity is strictly dependent and tightly regulated by heme. Heme plays key roles in oxygen sensing and utilization, inducing expression of many genes encoding respiratory functions, all of which are mediated by Hap1 (Zitomer and Lowry, 1992; Mense and Zhang, 2006).

HAP may also be a target of SNF1 activation via Rds2 (regulator of drug sensitivity), a zinc cluster transcription factor that is hyperphosphorylated by Snf1 kinase, although HAP can induce its target genes independent of SNF1 activity (Broach, 2012). After shifting from glucose to ethanol, Rds2 increases expression by binding to respiration gene promotors including Hap4 (Soontorngun et al., 2007). Recently, a novel complex Hap2/3/5-Gln3 has been suggested to form. In this case, Gln3 (glutamine metabolism), a transcription factor under TOR pathway, provides the activation domain (Hernández et al., 2011). This suggests competitive cross-talk between Gln3 and nitrogen source sensing versus glucose-depletion sensing of Hap4. In yeast, increased HAP

activity extends life span (Lin et al., 2002). *HAP4* overexpression (and thus induction of HAP target genes) extends replicative life span and increases mitochondrial respiration, which may mimic CR-mediated life span extension (Lin et al., 2002). Also, both CR- and amino acid starvation-induced retrograde response (RTG) and TCA cycle gene expression is in part due to upregulation of the *HAP4* gene (Wang et al., 2010).

Sch9

Sch9 is a serine/threonine kinase homolog of mammalian PKB (protein kinase B), S6 kinase/Akt. Sch9 has activating phosphorylations from various sensing pathways, including glucose, sphingolipid, and nitrogen sensors. Active Sch9 activates ribosome biogenesis, mass accumulation and cell growth (Roosen et al., 2005; Smets et al., 2008; Conrad et al., 2014). Pkh1/2 (PKB-activating kinase homolog 1/2) are serine/threonine kinase homologs of mammalian 3-phosphoinositide-dependent protein kinase 1 (PDK1) (Casamayor et al., 1999) that are activated by sphingolipids (Jacinto and Lorberg, 2008; Broach, 2012). Activated Pkh1/2 phosphorylate Sch9, which is required for initial Sch9 activation (Voordeckers et al., 2011).

Sch9 activation via glucose sensing pathways remains unclear. Sch9 and PKA have overlapping substrate specificity. In fact, overexpression of Sch9 can compensate for strains lacking PKA, but how Sch9 senses glucose is still unclear. Inactivated Sch9 does not reduce glucose-induced changes in transcription, whereas inactivation of PKA reduces it by 75%. Thus Sch9 may not be required for most glucose response; however in the absence of PKA, Sch9 is responsible for the remaining glucose response (Zaman et al., 2009; Broach, 2012). Sch9 may also cross-regulate the PKA pathway, via localization and phosphorylation of the PKA regulatory subunit, Bcy1. In addition, Sch9 can be activated by another glucose responsive pathway, the AMPK homolog, SNF1, which phosphorylates and enhances the activity of Sch9 (Lu et al., 2011). Not only can glucose-responsive pathways activate Sch9, but also in the presence of preferred nitrogen sources, Sch9 can be directly phosphorylated and activated by TOR. Sch9 is responsible for many cellular phosphorylation changes under TOR pathway (Urban et al., 2007), including stress response via inhibitory phosphorylation and cytosolic sequestration of Rim15 (Wanke et al., 2008) (see the subsection "PKA" for Rim15). Collectively, PKA, TOR and Sch9 regulate ribosome biogenesis, cell metabolism and stress response.

Mutations and deletion of Sch9 was shown to increase life span in both budding and fission yeast (Fabrizio et al., 2001; Kaeberlein et al., 2005b; Zuin et al., 2010). In budding yeast, both replicative and chronological life span have been shown to increase with reduced Sch9 activity (Fabrizio et al., 2001; Kaeberlein et al., 2005b; Longo and Fabrizio, 2012). Decreased Sch9 activity also led to increased stress resistance. Chronological life span increase by mutated Sch9 required stress-resistance transcription factors, Msn2/4 and Rim15 (Fabrizio et al., 2001). (See the subsection "PKA" for Msn2/4 and Rim15). Reduced Sch9 may also increase chronological life span in a reduced TOR activity dependent manner (Longo and Fabrizio, 2012).

TOR and nitrogen source sensing pathways

Yeast cells can sense nitrogen sources through the target of rapamycin (TOR) pathway. TOR is activated by preferred nitrogen sources such as glutamine, ammonium and certain amino acids. Although well studied, the exact mechanism of nitrogen source activation is still unclear. Reduction of TOR activity has been linked to increased yeast life span and CR (Kaeberlein et al., 2005b; Wei et al., 2008). In this section, we introduce TOR, its complexes, TORC1 and TORC2, and its cross-talk with other pathways.

Yeast TOR complex I (TORC1) functions as a protein kinase complex and consists of either Tor1 or Tor2, Kog1 (homolog of mammalian raptor), Lst8 (contains WD-repeat; required for general amino acid permease, Gap1, transport to the cell surface) and Tco89 (Tor complex one; regulates global H3 K56 acetylation). Tor1 and Tor2 are 67% identical in sequence and are highly similar to mammalian TOR protein. They are founding members of the phosphatidyl inositol 3′ kinase related kinases (PIKK), but do not have lipid kinase activity (Keith and Schreiber, 1995). TORC2 (TOR complex 2) complex contains exclusively Tor2, Avo1-3 (adheres voraciously to TOR2), Bit61 (binding partner of Tor2), and Lst8 (Loewith et al., 2002; Wedaman et al., 2003). TORC1 is sensitive to rapamycin inhibition, whereas TORC2 is not (Loewith et al., 2002). TORC2 mainly affects actin cytoskeleton organization via serine/threonine protein kinase Ypk1/2 (yeast protein kinase), lipid synthesis and cell survival (Loewith et al., 2002; Kamada et al., 2005; Niles and Powers, 2014). Recent studies have also connected TORC2 to amino acid starvation induced autophagy (Vlahakis et al., 2014; Vlahakis et al., 2014) and genome stability (Shimada et al., 2013). In this section, we will focus on the downstream targets of TORC1.

Recent studies have shown that the lysosome/vacuole membrane is the main location for activation of TORC1 signaling (Urban et al., 2007; Sturgill et al., 2008; Binda et al., 2009). In yeast, activity of TORC1 is regulated at the vacuolar membrane through interplay with the conserved EGO complex (EGOC), containing Ego1, Ego3, Gtr1, and Gtr2. Ego1, homologous to Ego3, is N-terminally myristoylated and palmitoylated, tethering EGOC to the vacuolar membrane (Dubouloz et al., 2005; Binda et al., 2009; Zhang et al., 2012). Gtr1 and Gtr2 are Ras-family GTPases (Sancak et al., 2008; Efeyan et al., 2012). Activated EGOC stimulates TORC1 via physical interaction in response to amino acids. In

mammals, EGOC appears to trigger TORC1 relocalization from the cytoplasm to the vacuolar membrane, however, detailed mechanisms remain unclear. *S. cerevisiae* TORC1 seems to constantly associate with the vacuolar membrane regardless of the amino acid level (Binda et al., 2009).

TORC1 controls various cellular processes such as nitrogen catabolite repression (NCR), amino acid transport, retrograde and stress response, and cell growth mainly by two major effector branches: Sch9, and the Tap42-PPase (Tap42: two A phosphatase associated protein; PPase: protein phosphatase) complex (Loewith et al., 2002; Urban et al., 2007; Broach 2012; Conrad et al., 2014). When TORC1 is activated, it phosphorylates and activates Sch9, leading to increased ribosome biogenesis, mass accumulation and cell growth (see the subsection "Sch9") (Urban et al., 2007). Activated TORC1 also phosphorylates Tap42, which leads to binding of TORC1 to the Tap42-PPase complex, resulting in inhibition of this complex. TORC1 and Tap42-PPase work cooperatively to regulate the phosphorylation status of many downstream targets (Fig. 1): activated TORC1 phosphorylates downstream targets and these phosphorylations are later removed by Tap42-PPase. TORC1 and Tap42-PPase also independently regulate different downstream targets by working with other kinases and phosphatases.

The main Tap42-PPase complex that regulates NCR, amino acid transport, retrograde and stress response is Tap42-PP2A (PP2A: protein phosphatase 2A) complex. When preferred nitrogen sources are present, inhibition of this complex by activated TORC1 prevents Tap42-PPA2 from accessing and dephosphorylating downstream targets. For example, Gln3 is a major transcription factor required for the induction of NCR genes in the absence of preferred nitrogen sources. When preferred nitrogen sources are present, Gln3 is heavily phosphorylated and kept in the cytoplasm via binding to its cytoplasmic sequestering partner, Ure2 (Blinder et al., 1996), and thus NCR genes are not expressed. During nitrogen limitation, TORC1 is inactive, which frees up Tap42-PP2A to access Gln3, leading to reduced phosphorylation, nuclear localization of Gln3 and NCR gene expression (Beck and Hall, 1999). The Tap42-PP2A complex also controls retrograde (RTG) response by regulating the nuclear localization of the Rtg1/3 transcription factors. Activated TORC1 phosphorylates the negative RTG regulator, Mks1. Phosphorylated Mks1 associates with 14-3-3 proteins (Bmh1/2) and this complex prevents nuclear localization of Rtg1/3. When TORC1 is inactive, Tap42-PP2A is activated, which dephosphorylates Mks1, leading to nuclear localization of Rtg1/3 and RTG gene expression. Rtg2 promotes Rtg1/3 nuclear localization by competing with 14-3-3 for binding with Mks1 (Mks1 is inactive when bound to Rtg2) (Dilova et al., 2004; Conrad et al., 2014). TORC1 and Tap42-PP2A also regulate stress response by regulating the phosphorylation status of Rim15 and its nuclear localization together with 14-3-3 proteins (Bmh1/2) (see the subsection "PKA"). TORC1 controls specific amino acid transport mainly via Npr1 (nitrogen permease reactivator). Npr1 is a TORC1 responsive protein kinase that regulates plasma membrane sorting of amino acid permeases (AAPs) such as Gap1 and Tat2 (tryptophan amino acid transporter). Gap1 is the general AAP, which mediates amino acid transport during amino acid (or nitrogen) starvation. In contrast, many other AAPs (such as Tat2) are only expressed or stabilized at the plasma membrane when preferred amino acids are present (see the subsection "SPS"). Activated TORC1 phosphorylates Npr1, leading to degradation of Gap1 (via enhancing its endocytosis) and stabilization of Tat2 (by inhibiting its endocytosis). In response to nitrogen or amino acid starvation, dephosphorylation of Npr1 (possibly by Tap42-Sit4, Sit4: a type 2A-related PPase) leads to stabilization of Gap1 on the plasma membrane and enhances the degradation of Tat2 (Broach 2012; Ljungdahl and Daignan-Fornier, 2012).

Amino acid sensing pathways

Yeast cells can sense amino acid sources through the GAAC (general amino acid control) and SPS (Ssy1-Ptr3-Ssy5) pathways (Fig. 1). Reduction of amino acid uptake has been shown to increase life span and linked to CR-mediated life span extension (Jiang et al., 2000; Powers et al., 2006; Fabrizio and Longo, 2007). In this section we introduce GAAC and SPS pathways, and their cross-talk with other pathways.

GAAC

In response to amino acid starvation, the general amino acid control (GAAC) regulatory system induces enzymes across all amino acid biosynthesis pathways (Marzluf 1997; Natarajan et al., 2001; Hinnebusch and Natarajan, 2002; Hinnebusch 2005; Ljungdahl and Daignan-Fornier, 2012). Amino acid starvation leads to uncharged tRNAs (Zaborske et al., 2009; Zaborske et al., 2010). GAAC inhibits overall protein synthesis and activates amino acid biosynthesis by allosteric activation of Gcn2 (general control nonderepressible) kinase: binding of uncharged tRNAs to a histidyl-tRNA synthetase-like domain on Gcn2 (Wek et al., 1989; Dong et al., 2000; Dever and Hinnebusch, 2005). Activated Gcn2 then phosphorylates Ser51 of the translation initiation factor, eIF2, leading to a reduction in the rate of ternary complex (TC: eIF2-GTP-Met-tRNAi, required for translation initiation) formation, which subsequently decreases overall protein synthesis (Wek et al., 1989; Dong et al., 2000; Dever and Hinnebusch, 2005). Gcn2-activated amino acid biosynthesis gene expression is mediated by regulating the translation of Gcn4 (general control nonderepressible), a leucine-zipper transcription factor. Gcn4 is the effector of GAAC and is required for inducing the expression of amino acid biosynthesis genes (Hinnebusch and Natarajan, 2002; Hinnebusch

2005). The mRNA of Gcn4 contains four small open reading frames in the 5′-untranslated region (uORF). When a scanning 40S ribosome with a TC encounters the first start codon of uORF1, the GTP bound to TC is hydrolyzed to GDP. Next, eIF2-GDP is released and the 60S ribosome is recruited to start translation. Translation terminates at the stop codon of uORF1, and 60S ribosome disassociates. The 40S ribosome continues to scan Gcn4 mRNA until it reacquires a TC to regain competency. When TC is high (no starvation), the 40C ribosome can reacquire TC and initiate translation at uORF2, uORF3 and then uORF4. Translation of uORF4 hinders the translation initiation at the start codon of Gcn4 resulting in no Gcn4 translation (and no induction of amino acid biosynthesis gene expression). When TC is low (under starvation conditions), after translation initiation at uORF1, the scanning 40S ribosome is more likely to reacquire TC after passing uORF2, uORF3 and uORF4, allowing translation initiation at the start codon of Gcn4. Gcn4 binds to promoters with the upstream activating sequence Gcn4 protein recognition element (UAS_{GCRE}) motif, GAGTCA, and activates amino acid biosynthesis genes (Hinnebusch and Natarajan, 2002; Hinnebusch 2005; Ljungdahl and Daignan-Fornier, 2012). Starvation also decreases proteasomal degradation of Gcn4 (Kornitzer et al., 1994; Ljungdahl and Daignan-Fornier, 2012).

GCN4 is also induced when NCR is derepressed (Fig. 1) (Godard et al., 2007; Ljungdahl and Daignan-Fornier, 2012). As discussed in the section "TOR", with non-preferred nitrogen sources, TORC1 no longer inhibits Tap42-PP2A, leading to increased NCR gene expression. When TORC1 is not active, Sit4, a type 2A-related PPase which is inhibited by active TORC1-Tap42-PP2A complex, dephosphorylates and activates Gcn2 (Cherkasova and Hinnebusch, 2003; Conrad et al., 2014). Therefore, decreased TORC1 activity increases Gcn4 via Sit4 and Gcn2. Gcn4 has also been found to act with Gln3 for induction of genes during rapamycin treatment (Staschke et al., 2010; Conrad et al., 2014). Snf1 has a complicated relationship with GAAC. During glucose depletion, which causes rapid translation inhibition (see details in the subsection "SNF1"), Snf1 also inhibits translation of Gcn4 and subsequent gene expression by Gcn4 (Conrad et al., 2014). However, in the presence of glucose and low amino acid conditions, Snf1 stimulates and activates Gcn2, leading to Gcn4 translation whereas in amino acid rich and low glucose conditions, Snf1 instead inhibits Gcn2 by promoting phosphorylation or inhibiting dephosphorylation of Gcn2 by Sit4 and Glc7 (Shirra et al., 2005; Shirra et al., 2008). Thus Snf1 both helps inhibit Gcn2 when GAAC is off, and helps stimulate Gcn2 when GAAC is on, depending on the presence of glucose.

SPS

Thus far in terms of amino acids and nitrogen sources we have discussed internal sensing pathways. The pathway responsible for external amino acid sensing is the SPS (Ssy1-Ptr3-Ssy5) amino acid nutrient-sensing pathway (Abdel-Sater et al., 2011; Omnus et al., 2011). Ssy1 is a plasma membrane broad range amino acid permease-like sensor with no transport activity. Ssy1 is in complex with the phosphoprotein, Ptr3, and the serine protease, Ssy5. Upon formation, Ssy5 autocleaves into a catalytic domain and a prodomain, which remain associated until signaling is activated. In the presence of external amino acids, Ssy1 sends a signal via conformational changes that triggers subsequent phosphorylations of Ptr3 and Ssy5 by the kinases, Yck1 and Yck2 (also in the subsection "Snf3-Rgt2") (Liu et al., 2008; Omnus et al., 2011). Once phosphorylated, the prodomain and catalytic domain of Ssy5 disassociate. The phosphorylated Ssy5 prodomain is ubiquitinated by Grr1 and marked for proteasomal degradation. The Ssy5 catalytic domain then cleaves the N-terminal cytoplasmic retention domain of both SPS pathway transcription factors, Stp1 and Stp2 (Andréasson et al., 2006; Omnus et al., 2011; Omnus and Ljungdahl, 2014). Without the cytoplasmic retention domain, Stp1 and Stp2 can bypass the surveillance of inner nuclear membrane ubiquitin ligase Asi (amino acid sensor-independent) complex components, Asi1, Asi2 and Asi3, entering the nucleus to modulate SPS pathway downstream gene expression such as increasing amino acid permease expression and decreasing amino acid biosynthesis (Zargari et al., 2007; Foresti et al., 2014; Omnus and Ljungdahl, 2014). SPS amino acid sensing pathway and Snf3-Rgt2 glucose sensing pathway have much in common. Both require Yck1/2 phosphorylation and Grr1 ubiquitination for activation. Ssy1, Snf3 and Rgt2 are all transporter-like sensors with long-terminal tails and no transporter function (Ljungdahl 2009; Rubio-Texeira et al., 2010; Broach 2012; Ljungdahl and Daignan-Fornier, 2012; Conrad et al., 2014). Both sensors also directly activate their transcription factors to induce changes in gene expression targets. Recently in yeast, decreased SPS pathway activity was shown to extend replicative life span (Tsang et al., 2015), which was further increased by CR. SPS-induced life span extension required components of the malate-pyruvate NADH shuttle and partially required Pho8, a vacuolar phosphatase. Decreased SPS activity also increased phosphate sensing (*PHO*) pathway expression, thus active SPS likely has a repressive effect on *PHO* pathway components (Tsang et al., 2015) (see the section "Phosphate sensing pathway" for cross-talk).

Phosphate sensing pathway

In yeast, inorganic phosphate (Pi) is sensed by the phosphate-responsive signaling (*PHO*) pathway. Recently, *PHO* pathway was linked to life span: Pho8, a target of *PHO* pathway, was partially required for reduced SPS-mediated life span

extension (Tsang et al., 2015). In this section we will discuss Pi sensing and response by the *PHO* pathway and cross-talk with other pathways.

Pi sensing, acquisition, and storage are mainly mediated by the *PHO* pathway that controls Pi transporters, regulatory factors, and effectors (Lenburg and O'Shea, 1996; Persson et al., 2003; Auesukaree et al., 2004). When Pi is highly available, *PHO* signaling downstream targets are repressed by the cyclin-dependent kinase complex, Pho80-Pho85 (Pho80: cyclin, Pho85: cyclin dependent kinase), which phosphorylates and inactivates the major transcription factor, Pho4, by preventing its nuclear localization. Upon Pi limitation, the synthesis of inositol heptakisphosphate (IP7) increases (Auesukaree et al., 2005; Lee et al., 2007). IP7 was shown to allosterically bind to the tertiary complex of Pho81 (a cyclin-dependent kinase inhibitor)-Pho80-Pho85, inducing a conformational change of Pho81 that prevents phosphorylation of Pho4 by Pho85 (Lee et al., 2008). Unphosphorylated Pho4 accumulates in the nucleus, and along with transcription factor, Pho2, activates *PHO*-responsive genes whose expression products include high affinity Pi transporters (Pho84 and Pho89), repressible phosphatases (Pho5 and Pho8), and factors for phosphate mobilization from vacuolar polyphosphate storage.

Pi sensing also affects the cell cycle: Cln3 (G_1 cyclin) is a target for phosphorylation by Pho80-Pho85 (Menoyo et al., 2013). Under Pi starvation, Pho80-Pho85 is inactive and Cln3 is dephosphorylated, which leads to proteasomal degradation and cell cycle arrest. Repletion of Pi requires and activates Pho80-Pho85 to phosphorylate and activate Cln3. This allows cells to progress over start in G_1 and re-enter the cell cycle (Menoyo et al., 2013).

Activation of *PHO* pathway also concomitantly increases salvaging of nicotinamide riboside (NR), a precursor to NAD^+, since certain phosphatases such as Pho5 and Pho8 can utilize NAD^+ intermediate NMN as a substrate, producing NR while removing Pi (Lu et al., 2011). It is not yet fully understood how cells sense the level of available Pi to elicit proper metabolic responses. Interestingly, the *pho84Δ* mutant has constitutive expression of *PHO5* (Ueda and Oshima, 1975; Bun-Ya et al., 1991). The defect in Pi transport caused by *PHO84* mutations resulted in a low level of intracellular Pi, leading to constitutive derepression of the *PHO* pathway and its downstream genes (Wykoff and O'Shea, 2001; Auesukaree et al., 2004). In addition to Pi availability, some studies show that the *PHO* signaling pathway also responds to changes in intermediate metabolites of the purine synthesis pathway in an IP7-independent manner (Gauthier et al., 2008; Pinson et al., 2009).

Phosphate signaling has also been linked to glucose sensing pathways. For example, after prolonged Pi starvation, repletion of Pi to cells leads to PKA activation-dependent degradation of phosphate transporter, Pho84 (Giots et al., 2003; Mouillon and Persson, 2005; Lundh et al., 2009; Popova et al., 2010; Samyn et al., 2012). These studies support a role for Pho84 as a Pi transceptor, a sensor and transporter of Pi. Also, other components in the *PHO* pathway have been associated with glucose metabolism (Kang et al., 2014; Kang et al., 2014). Phosphate sensing *PHO* pathway and glucose sensing PKA pathway also have similar effects on the transcription factor, Rim15 (see the subsection "PKA"). Both PKA and Pho80-Pho85 complex phosphorylate and inhibit Rim15. Phosphorylated Rim15 is anchored to the cytoplasm by 14-3-3 (Bmh1/2) proteins (Wanke et al., 2005). Active Rim15 increases expression of stress response and post diauxic shift genes via Msn2/4 and Gis1, respectively (Lee et al., 2013). Active Msn2/4 also increases Pnc1 activity (see the subsection "PKA"), which increases genome stability and NAD^+ metabolism. Thus *PHO* pathway may regulate NAD^+ metabolism (see the subsection "*Other nutrient sensing pathways and NAD^+ metabolism*") via increased NR salvaging, as previously mentioned, and via this Rim15-Msn2/4-Pnc1 pathway.

As previously mentioned in the subsection "SPS", another point of cross-talk is between phosphate sensing *PHO* pathway and extracellular amino acid sensing SPS pathway. Decreased SPS pathway activity increases *PHO* pathway activity: increased overall expression of *PHO* pathway, including the *PHO* pathway transcription factor, Pho4, and slightly increased activity of repressible vacuolar phosphatase, Pho8. Also, expression of Pho8, increases under reduced SPS activity, independent of the canonical *PHO* transcription factors Pho2 and Pho4. Full SPS-mediated life span extension also required Pho8 (Tsang et al., 2015). Thus components of *PHO* pathway may also be activated under other conditions or sensor pathways.

Factors contributing to life span extension

For decades the budding yeast, *Saccharomyces cerevisiae*, has been a model organism for uncovering genes that regulate life span. Studies in model systems including yeast, demonstrate that single gene mutations can alter longevity (Jazwinski 2000; Kenyon 2001; Dilova et al., 2007; Longo and Fabrizio, 2012). Additionally, CR extends life span in a variety of species, further supporting conservation of longevity-regulating pathways (Weindruch and Walford, 1998). CR also delayed or reduced the incidence of many age-associated diseases such as cancer and diabetes (Weindruch and Walford, 1998; Guarente, 2013). Like CR, mitigated nutrient sensing activity generally leads to life span extension. *S. cerevisiae* has contributed considerably to aging and CR studies, and helped pave the way for further research in metazoans. Although the molecular mechanisms underlying life span extension still remain unclear, as more longevity factors are identified, it is evident that aging is modulated by the complex interplay of signaling pathways at both the cellular and organismal level.

Yeast life span is studied in two ways: replicative life span

(RLS) and chronological life span (CLS). RLS measures total cell divisions an individual yeast cell undergoes before senescence (division potential) (Mortimer and Johnston, 1959; Jazwinski, 1990), whereas CLS measures the time cells remain viable at a non-dividing state (post-mitotic survival) (Fabrizio and Longo, 2003; Longo and Fabrizio, 2012). The mother-daughter cell asymmetry in *S. cerevisiae* can be easily observed under the microscope, which allowed development of the RLS assay (Mortimer and Johnston, 1959). Although RLS and CLS define two very distinct forms of longevity, many longevity factors appear to regulate both CLS and RLS.

Generally, reduced nutrient signaling can increase life span, for example CR. In yeast, moderate CR is imposed by reducing the glucose in rich media from 2% to 0.5% (Lin et al., 2000; Easlon et al., 2008; Wei et al., 2008). Under this CR condition, growth rate remains robust and both RLS and CLS extend. Moderate CR shunts carbon metabolism from fermentation toward the mitochondrial TCA cycle, with a concomitant increase in respiration (Lin et al., 2002). In yeast, moderate CR is suggested to extend life span through reducing the activities of conserved nutrient-sensing pathways such as the PKA, Sch9 and TOR kinase pathways (Lin et al., 2000; Fabrizio et al., 2001; Kaeberlein et al., 2005b). Decreased activity of the glucose sensing PKA pathway extends both RLS and CLS (Sun et al., 1994; Lin et al., 2000; Fabrizio et al., 2001). Also, deleting the nutrient responsive Sch9 (*sch9*Δ) and nitrogen source sensing Tor1 (*tor1*Δ) kinases extend both RLS and CLS (Fabrizio et al., 2001; Longo 2003; Kaeberlein et al., 2005b), supporting that reduced nutrient sensing activity can extend life span. Notably, mutants of the PKA, TOR and Sch9 pathways have been suggested to genetically mimic CR. However, some nutrient sensing pathways can modulate life span independent of the CR pathway. For example, deleting components of the amino acid sensing SPS pathway extends RLS and CR further extends the long life span of these SPS mutants (Tsang et al., 2015).

In addition to nutrient sensing pathways, other longevity factors were identified in yeast, some of which have also been linked to CR. These factors include proteins that modulate mitochondrial function (Barros et al., 2004; Bonawitz et al., 2007; Scheckhuber et al., 2007; Veatch et al., 2009; Ocampo et al., 2012; Erjavec et al., 2013), stress response/hormesis/mitohormesis (Bonawitz et al., 2007; Mesquita et al., 2010; Pan 2011; Pan et al., 2011; Longo and Fabrizio, 2012; Ocampo et al., 2012), NAD^+-dependent deacetylase Sir2 family activity (Imai and Guarente, 2014) (discussed later), asymmetric partitioning of damaged proteins (Erjavec and Nyström, 2007; Erjavec et al., 2007, 2008), genome stability (Weinberger et al., 2007; Andersen et al., 2008; Unal et al., 2011), homeostasis of NAD^+ and other metabolic factors (Lin et al., 2000; Anderson et al., 2002; Belenky et al., 2007; Matecic et al., 2010; Kato and Lin, 2014a) (see the section "NAD^+ biosynthetic and signaling pathways in yeast"), vacuolar function (Fabrizio et al., 2010; Hughes and Gottschling, 2012), ribosome biogenesis (Steffen et al., 2012), cell size/hypertrophy (Biliński and Bartosz, 2006; Yang et al., 2011; Biliński et al., 2012), and regulation of proteostasis (Kruegel et al., 2011; Delaney et al., 2013; Schleit et al., 2013; Yao et al., 2015). Additionally, acetic acid released into the growth medium as a metabolic byproduct during chronological aging was suggested to be the major factor that shortens CLS. Although pH neutralization was shown to offset acetic acid induced toxicity and protect both CLS and RLS (Burtner et al., 2009; Murakami et al., 2011; Murakami et al., 2012), other studies showed that acetic acid and pH are not the only determinants of CLS (Longo and Fabrizio, 2012; Wu et al., 2013).

Variations in CR protocols such as amino acid limitation and/or further carbon source reduction/substitution have also been implemented (Jiang et al., 2000; Kaeberlein et al., 2005a; Fabrizio and Longo, 2007; Longo and Fabrizio, 2012). In yeast, the role of Sir2 in CR is highly debated: RLS extension by moderate CR (0.5% glucose) (Lin et al., 2000; Kaeberlein et al., 2002; Lamming et al., 2005; Easlon et al., 2007; Smith et al., 2007; Easlon et al., 2008; Wei et al., 2008) requires both NAD^+ and Sir2 (Lin et al., 2000), whereas RLS extension by more severe CR (0.05% glucose) does not require Sir2 (Kaeberlein et al., 2004; Easlon et al., 2007). In fact, without the Sir2 family, severe CR further extends RLS (Easlon et al., 2007). Akin to Sir2 in CLS: under nutrient deprivation, deleting *SIR2* further extends CLS in certain genetic backgrounds (Fabrizio et al., 2005). Cells likely respond differently to each CR regimen. Perhaps severe CR additionally triggers gluconeogenesis, which is activated upon glucose starvation. Gluconeogenesis may also be induced in nutrient deprived *sir2*Δ mutants that further extend CLS (Fabrizio et al., 2005). A key gluconeogenesis enzyme, Pck1, is a Sir2 substrate, thus in *sir2*Δ mutant, Pck1 remains active and promotes gluconeogenesis (Lin et al., 2009). Yet, age-enhanced gluconeogenesis has been shown to shorten CLS and homologues of Sir2, Hst3 and Hst4, downregulate age-enhanced gluconeogenesis (Hachinohe et al., 2013). Moreover, human Sirt1 has been reported to deacetylate PGC1-α, which induces gluconeogenesis (Rodgers et al., 2005). Together, these studies demonstrate the complex roles of Sir2 in CR, a complexity typical of metabolic longevity factors.

NAD^+ biosynthetic and signaling pathways in yeast

Pyridine nucleotides are in a myriad of cellular processes. In yeast, factors in the NAD^+ biosynthetic pathways have several roles. As previously mentioned, Sir2 and moderate CR require the longevity factor, NAD^+ (Lin et al., 2000). Also, NAD^+ metabolism is required for normal life span as well as life span extension by reduced SPS amino acid

signaling activity (Tsang et al., 2015). NAD$^+$ intermediates can be signaling molecules, and high intracellular concentrations can inhibit certain cellular processes. In this section we discuss the NAD$^+$ biosynthetic pathways in yeast and their roles in signaling.

Overview of NAD$^+$ biosynthesis

Cellular NAD$^+$ is synthesized from several precursor molecules. Eukaryotes utilize tryptophan to synthesis NAD$^+$*de novo* and also salvage NAD$^+$ intermediates such as nicotinamide (Nam), nicotinic acid (NA) and nicotinamide riboside (NR). 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. As shown in Fig. 2, quinolinic acid (QA) is mainly derived from tryptophan via the *de novo* pathway: five enzymatic reactions (Bna2, Bna7, Bna1, Bna4, Bna5) and a spontaneous cyclization (Bender 1983; Wogulis et al., 2008). QA is then converted into NaMN by phosphoribosyltransferase, Bna6, transfer of the phosphoribose moiety of phosphoribosyl pyrophosphate (PRPP) (Panozzo et al., 2002). *De novo* synthesis and NA/Nam salvaging pathways converge at NaMN. As part of NA/NAM salvage, another phosphoribosyltransferase, Npt1, converts NA into NaMN (Preiss and Handler, 1958a, 1958b). NA is mainly salvaged from Nam by Pnc1 deamidase, but the NA transporter, Tna1, also imports both NA and QA (Llorente and Dujon, 2000; Ohashi et al., 2013). Next, NaMN adenylyltransferases, (NaMNATs), Nma1 and Nma2, convert NaMN into nicotinic acid adenine dinucleotide (NaAD or deamido-NAD$^+$) by transfer of the AMP moiety from ATP. Amidation of NaAD by Qns1, a glutamine-dependent NAD$^+$ synthetase, completes NAD$^+$ synthesis (Bieganowski et al., 2003).

In the shortest branch of NR salvaging, NR is phosphorylated by Nrk1 to produce NMN (Bieganowski and Brenner, 2004; Belenky et al., 2007) and is subsequently adenylylated to NAD$^+$ by NMN adenylyltransferases (NMNATs), Nma1, Nma2 and Pof1 (Natalini et al., 1986; Emanuelli et al., 1999; Garavaglia et al., 2002; Emanuelli et al., 2003; Magni et al., 2004; Kato and Lin, 2014). Nrk1 can also convert nicotinic acid riboside (NaR), a deamidated form of NR, to NaMN (Belenky et al., 2007). Unlike higher eukaryotes including mammals, yeasts do not possess Nam phosphorybosyltransferase (Nampt), the enzyme that converts Nam to NMN. Instead, Nam is deamidated to NA by Pnc1, and shunted into NA/NAM salvaging (Ghislain et al., 2002). The newer, longer branch of NR salvaging connects NR to NA/Nam salvaging, and includes nucleosidases Pnp1, Urh1, and Meu1, which convert NR to Nam, or NaR to NA (Belenky et al., 2007). Furthermore, the nucleotidases, Isn1 and Sdt1 (Bogan et al., 2009), and phosphatases, Pho5 and Pho8 (Lu et al., 2009; Lu et al., 2011), contribute to NR metabolism by converting NMN to NR.

Yeast cells produce NAD$^+$ predominantly from the NA/Nam salvage pathway during exponential growth (Sporty et al., 2009), due to niacin (NA and Nam)-rich standard media and very efficient NA transport by Tna1 (Llorente and Dujon, 2000). Also, NR is an efficient NAD$^+$ precursor: contributing up to ~50% of the NAD$^+$ pool and supporting NAD$^+$-dependent reactions (Bieganowski and Brenner, 2004; Belenky et al., 2007). Although standard media is NR-free, NR is an endogenous metabolite that is dynamically released into and retrieved from the environment (Lu et al., 2009). Extracellular NR is imported by the high-affinity NR transporter, Nrt1 (Belenky et al., 2008). Perhaps a flexible NR/NAD$^+$ pool facilitates prompt adjustments in response to metabolic stresses (Lu and Lin, 2010). Indeed, NR salvaging activity was essential for maintaining NAD$^+$ homeostasis, cellular fitness and life span (Bogan et al., 2009; Lu et al., 2009).

Additional roles of NAD$^+$ intermediates and biosynthetic enzymes

Intermediates of NAD$^+$ biosynthesis can also function as signaling molecules. Intracellular concentrations of many NAD$^+$ intermediates are maintained at low levels, characteristic of signaling molecules (Evans et al., 2010), or compartmentalized (see the subsection "Compartmentalization of NAD$^+$ metabolism"). We previously discovered that low NaMN level is associated with activation of the *PHO* pathway (Lu et al., 2011). *PHO*-regulated Pho5 activity increased specifically in *npt1Δ* mutant (Npt1 is the major NaMN producer), suggesting that depletion of NaMN leads to *PHO* activation (Lu et al., 2011). Activated *PHO* pathway leads to increased transport and mobilization of Pi with concomitant increase in NR levels and thus boosts NAD$^+$ metabolism (see the section "Phosphate sensing pathway"). Moreover, nicotinic acid adenine dinucleotide phosphate (NAADP) and cyclic ADP-ribose (cADPR), function as signaling molecules to regulate calcium homeostasis in a variety of organisms (Clapper et al., 1987; Guse and Lee, 2008). CD38, an ADP-ribosylcyclase, has been shown to produce NAADP and cADPR from NADP$^+$ and NAD$^+$, respectively (Graeff et al., 2006; Li et al., 2013). Other NAD$^+$ intermediates, such as NR and NMN, may also function as signaling molecules to regulate NAD$^+$ homeostasis or cellular processes (Kato and Lin, 2014). We recently showed that most NR is vacuolar and NMN may be sequestered by Pof1 binding, leading to lower levels of free NR and NMN (Kato and Lin, 2014b).

Additionally, it may be crucial to maintain low levels of intracellular NAD$^+$ intermediates since high concentrations can inhibit certain cellular processes. For example in bacteria, NMN is a product and inhibitor of NAD$^+$-dependent DNA ligase, thus NMN deamidase is suggested to maintain low levels of intracellular NMN (Cheng and Roth, 1995). In yeast, we recently showed that NMN is constantly converted into

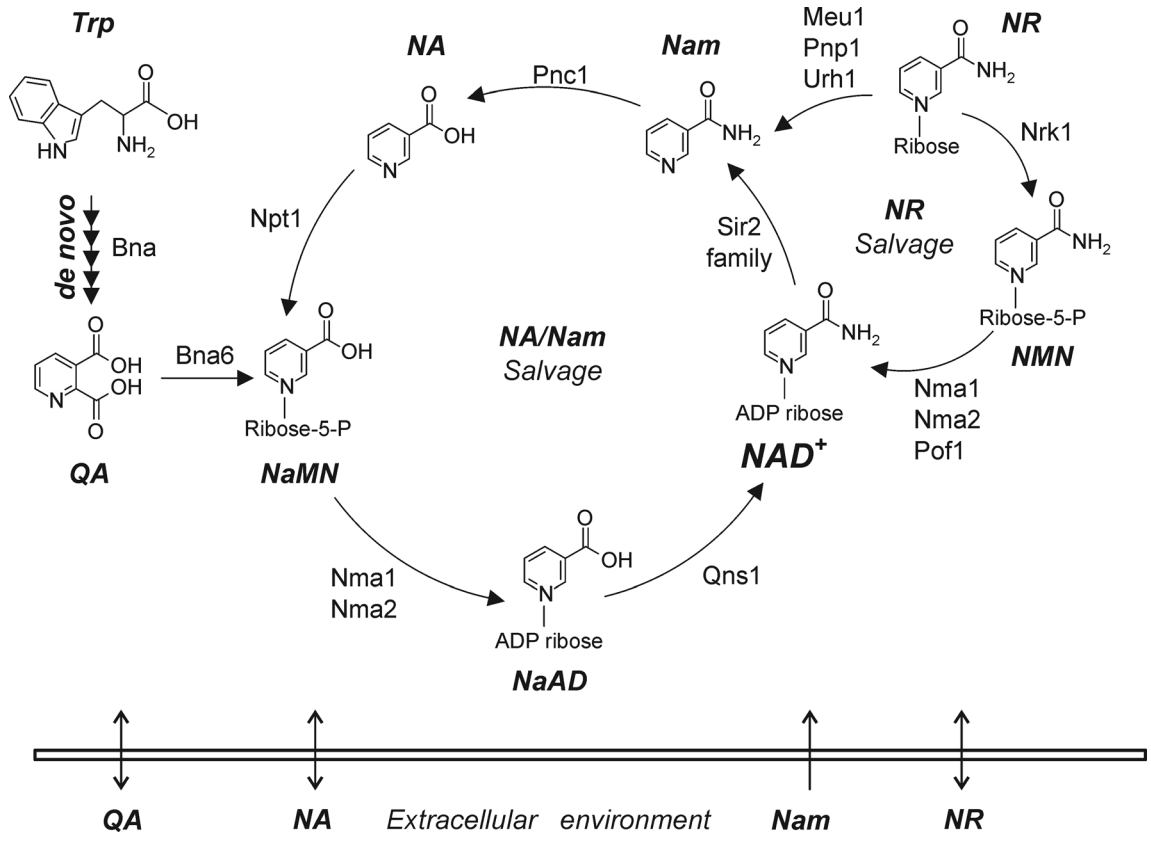

Figure 2 NAD^+ biosynthetic pathways in *S. cerevisiae*. NAD^+ is synthesized from two key intermediates, nicotinic acid mononucleotide (*NaMN*) and nicotinamide mononucleotide (*NMN*), via the *de novo* and *NA/Nam/NR* salvaging pathways. *Trp*: tryptophan. *QA*: quinolinic acid. *NaMN*: nicotinic acid mononucleotide. *NaAD*: nicotinic acid adenine dinucleotide. NAD^+: nicotinamide adenine dinucleotide. *Nam*: nicotinamide. *NA*: nicotinic acid. *NR*: nicotinamide riboside. *NMN*: nicotinamide mononucleotide. Bna: Bna2, Bna7, Bna1, Bna4, Bna5. Bna6: phosphorybosyltransferase; transfers the phosphoribose moiety of phosphoribosyl pyrophosphate (PRPP). Nma1, Nma2: NaMN and NMN adenylyltransferase, transfers the AMP moiety from ATP. Qns1: a glutamine-dependent NAD^+ synthetase. Sir2 Family: Sir2, Hst1, Hst2, Hst3, Hst4. Pnc1: deamidase. Npt1: phosphorybosyltransferase. Meu1, Pnp1, Urh1: nucleosidases, can also convert nicotinic acid riboside (NaR) to NA (not shown). Nrk1: NR kinase, can also convert NaR to NaMN (not shown). Pof1: NMN adenylyltransferases. Not shown: Tna1, NA transporter, also imports QA. Nrt1, high-affinity NR transporter. Isn1, Sdt1, nucleotidases, and Pho5, Pho8 phosphatases, which convert NMN to NR.

NR, which is more mobile and readily excreted, re-assimilated or sent to storage (Kato and Lin, 2014b). The NAD^+ intermediate, QA, was also shown to be produced and excreted like NR (Ohashi et al., 2013). Additionally, Nam is a product and inhibitor of NAD^+-dependent deacetylase, Sir2, thus Nam clearance is critical for Sir2 activity and life span (Gallo et al., 2004) (see the subsection "Sir2 and NAD^+ metabolism"). Nam can be cleared by enzymes of the NA/NAM salvage pathways: in yeast by Pnc1, a Nam deamidase; in mammals by Nampt, a Nam phosphoribosyltransferase (Bitterman et al., 2002; Revollo et al., 2004). Nam is also methylated by the Nam methyltransferase, Nnt1, a homolog of human NNMT (N-nicotinamide methyltransferase) (Anderson et al., 2003; Lin et al., 2004), a modification that induces a hormetic response to protect against oxidative damage (Schmeisser et al., 2013). A recent study in *C. elegans* suggests that methylated Nam can induce a transient hormetic response, which leads to life span extension (Schmeisser et al., 2013). NADH was also reported to competitively inhibit Sir2 (Lin et al., 2004) (see the subsection "Sir2 and NAD^+ metabolism"). Thus, balancing the concentrations of pyridine nucleotides and their metabolites may be critical for regulating many cellular processes. Moreover, specific NAD^+ synthesis enzymes, such as NMNATs, have been shown to play roles in other cellular processes. *Salmonella* NadR is a multi-functional NMNAT with a role in transcriptional regulation (Grose et al., 2005). *Drosophila* NMNAT was shown to possess chaperone activity which helps protect axons (Zhai et al., 2008). In *S. cerevisiae*, Nma1 and Nma2 were shown to alleviate proteotoxicity in yeast models of proteinopathies (Ocampo et al., 2013). Overall, these studies demonstrate that specific NAD^+ intermediates as well as NAD^+ biosynthesis enzymes also play roles in other cellular processes, further highlighting

the importance of NAD+ homeostasis factors in maintaininFg cellular function. The next section further discusses the regulation of NAD+ metabolism and cross-talk with other signaling pathways.

Regulation of NAD+ metabolism

Sir2 and NAD+ metabolism

The Sir2 family proteins, sirtuins, are highly conserved from bacteria to humans (Brachmann et al., 1995; Frye, 2000), with a conserved core domain required for their enzymatic activity as NAD+-dependent protein deacetylases or ADP-ribosylases (North and Verdin, 2004). Sir2 was first identified in the Sir1/2/3/4 silencing complexes in yeast (Ivy et al., 1986; Rine and Herskowitz, 1987), which are required to maintain and repress three repetitive genomic regions: telomeres, the cryptic mating type loci (*HML* and *HMR*), and the rDNA loci (Hecht et al., 1996; Smith and Boeke, 1997; Strahl-Bolsinger et al., 1997; Moazed 2001). Each locus employs unique DNA binding factors that recruit specific Sir proteins to deacetylate histones, compacting chromatin configuration, thus mediating silencing. Among these Sir proteins, only Sir2 is required for silencing of all three regions, and the NAD+-dependence of Sir2 activity links cellular metabolic status to Sir2 complex-mediated transcriptional silencing (Imai et al., 2000; Landry et al., 2000; Smith et al., 2000). Sir2 homologs in yeast include Hst1, Hst2, Hst3 and Hst4 (homologous to Sir two), of which nuclear Hst1 is most homologous to Sir2 and when overexpressed suppresses silencing defects at the mating type loci of Sir2 deletion mutant (Brachmann et al., 1995). Hst1 may also mediate Sir complex-independent transcriptional regulation by interacting with the transcription regulator, Sum1 (Xie et al., 1999; Sutton et al., 2001). Although Hst1 is most homologous, Hst2 is the most abundant Sir2 homolog in yeast, accounting for the bulk of intracellular NAD+-dependent deacetylase activity (Smith et al., 2000). Hst2 appears to shuttle between the nucleus and cytoplasm to regulate rDNA and telomere silencing (Smith et al., 2000; Perrod et al., 2001; Halme et al., 2004; Lamming et al., 2005; Wilson et al., 2006). Nuclear Hst3 and Hst4 have also been linked to genome stability by regulating rDNA gene silencing and controlling histone H3 (K56) acetylation (Brachmann et al., 1995; Celic et al., 2006; Hachinohe et al., 2011).

Key Sir2 regulators include NAD+ and its derivatives, thus NAD+ levels affect Sir2 activity and its downstream events. Sir2-mediated processes are affected by mutations that cause deficiency in NAD+ production (see the subsection "Overview of NAD+ biosynthesis"). Deleting the NA phosphoribosyltransferase, *NPT1,* gene significantly reduces NAD+ levels, abolishes Sir2-mediated silencing (Smith et al., 2000) and CR-mediated life span extension (which requires both Sir2 and NAD+) (Lin et al., 2000). Nam, a product of Sir2-mediated deacetylation, is also a potent non-competitive inhibitor of Sir2 (Bitterman et al., 2002; Sauve and Schramm, 2003; Schmidt et al., 2004). Preventing Nam accumulation is important for Sir2 function since increasing Nam concentration in growth media or blocking Nam clearance by deleting the nicotinamidase, *PNC1* (Ghislain et al., 2002), reduced Sir2-mediated silencing and shortened life span (Bitterman et al., 2002; Gallo et al., 2004). Also, CR has been suggested to activate Sir2 by decreasing Nam accumulation (Bitterman et al., 2002). Interestingly, the NAD+ intermediate, NMN, could also inhibit Sir2 (Schmidt et al., 2004), therefore if NAD+ synthesis is not required, cells may maintain low levels of NMN (see the subsection "Additional roles of NAD+ intermediates and biosynthetic enzymes"). Another NAD+ derivative, NADH, was reported to competitively inhibit Sir2: lowering the NADH level by genetic modifications increased Sir2-mediated silencing and life span (Lin et al., 2004). However, NADH is a weak Sir2 inhibitor, and reported cellular NADH levels appear too low to inhibit Sir2 activity in vivo (Schmidt et al., 2004). Perhaps intracellular compartmentalization of NAD+, NADH and/or specific protein–protein interactions create locally high NADH levels (low NAD+/NADH ratio) which inhibit Sir2 in vivo, and when NADH levels are lowered, (higher NAD+/NADH ratio) Sir2 is activated. It has been suggested that Sir2 affinity/sensitivity toward its substrates and inhibitors varies when Sir2 is in complex with different interacting partners (Tanny et al., 2004).

Although regulated by NAD+ and its derivatives, Sir2 and Hst1 also regulate NAD+ metabolism. It was reported that *de novo* NAD+ synthesis is repressed by Hst1 and that *HST1* deletion raises the steady-state level of NAD+ (Bedalov et al., 2003). Hst1 has also been shown to repress the expression of NR transporter, Nrt1 (Li et al., 2013) and our previous studies suggested that Sir2 modulates the flux of NR salvage, since NR release increases in the *sir2*Δ mutant (Lu et al., 2009). Perhaps the Sir2 family preserves the NAD+ pool by producing Nam in deacetylation reactions, thus supporting NA/Nam salvaging (Fig. 2) (Anderson et al., 2002) and decreasing flux into the NR branch. Indeed, deleting *SIR2* further decreased the NAD+ pool in cells lacking NR assimilation activity (Lu et al., 2009). Still, the mechanisms remain unclear and we await future studies determining the molecular and biochemical roles of the Sir2 family in NAD+ metabolism.

Compartmentalization of NAD+ metabolism

In yeast, NAD+ synthesis occurs mainly in the cytosol and it is unclear how NAD+ and its derivatives are transported into or from various intracellular compartments, such as the mitochondria and vacuole. This section discusses how compartmentalization of NAD+ metabolism can regulate NAD+ homeostasis and cellular functions.

Mitochondrial compartmentalization of NAD^+(H)

In yeast, it is unclear how NAD^+ and its derivatives are transported into the mitochondria. When mitochondrial activity is high, NAD^+ is required for many of its reactions, but the mitochondrial inner membrane is not permeable to NAD^+ or NADH. NAD^+ may be transported into the mitochondria by the NAD^+ carrier proteins, Ndt1 and Ndt2 (Todisco et al., 2006), and other NAD^+ intermediate transporters may also exist. Our knowledge of mitochondrial NAD^+(H) homeostasis derives mostly from studying the NADH shuttle systems: groups of enzymes and carriers that move small or 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 (Bakker et al., 2001; Houtkooper et al., 2010). We previously showed that the CR-mediated respiration-induced increase in the mitochondrial NAD^+/NADH ratio (see the subsection "Sir2 and NAD^+ metabolism" for NAD^+/NADH ratio) can be transmitted to the cytosol by the malate-aspartate shuttle (Easlon et al., 2008). This balancing of NAD^+/NADH ratio between mitochondrial and cytosolic pools was required for CR, since deleting NADH shuttle components abolished CR-mediated life span extension (Easlon et al., 2008). Another pathway that requires NADH shuttles for life span extension is SPS signaling. In this system, a different NADH shuttle, the malate-pyruvate shuttle, plays a key role in reduced SPS-induced life span extension, (whereas the malate-aspartate shuttle is required for CR) (Easlon et al., 2008). Interestingly, although CR increases NAD^+/NADH ratio (Easlon et al., 2008), reduced SPS pathway does not. Although detailed molecular mechanisms remain uncharacterized, these studies suggest that increased flux between the mitochondrial and cytoplasmic NAD^+(H) pools due to NADH shuttles may confer metabolic flexibility to support various life span extending changes (Tsang et al., 2015). In support, rapid transmission of NAD^+/NADH may generally be important since overexpressing NADH shuttle components alone extended life span (Easlon et al., 2008). Also in mammals, impairments of mitochondrial metabolism and NADH shuttles have been implicated in age-associated diseases such as diabetes (Eto et al., 1999; Haigis et al., 2006). In summary, NADH shuttles play important roles in mitochondrial metabolism and metabolic fitness by regulating NAD^+ homeostasis. Identifying additional mitochondrial NAD^+ homeostasis factors will provide insight into the role of mitochondrial compartmentalization in NAD^+ homeostasis.

Vacuolar and cytosolic compartmentalization of NMN and NR

The vacuole plays an important role in NR and NAD^+ homeostasis. Vacuolar and mitochondrial function are also linked. We previously showed the vacuole is not only a major source of NR production, but also an intracellular NR storage compartment (Lu et al., 2011). There are two major intracellular pools of NR in yeast: vacuolar pool and cytosolic pool. Released NR level in the growth medium reflects the dynamic cytosolic NR pool and deleting cytosolic *ISN1* and *SDT1* (nucleotidases that produce NR) greatly decreases NR release (Bogan et al., 2009; Lu et al., 2011; Kato and Lin, 2014b). Whereas intracellular NR level reflects the stored (vacuolar) pool and deleting vacuolar *PHO8* largely decreases intracellular NR level (Bogan et al., 2009; Lu et al., 2011; Kato and Lin, 2014b). Pho8 produces NR from NMN (Lu et al., 2011), requires a vacuolar peptidase for activation (Noda and Klionsky, 2008), and is canonically activated by the *PHO* pathway. However, we recently showed *PHO*-independent activation of Pho8 in mutants with reduced SPS signaling activity (Tsang et al., 2015). Supporting a critical role for the vacuole in NR homeostasis, our previous genetic screens identified 31 mutants which had increased NR release that were deficient in either vacuolar ATP synthase (v-ATPase) assembly (Lu et al., 2011), (maintains vacuolar matrix acidity) or potentially related vesicle-mediated transport. Thus the vacuole and vesicle-mediated transport may play a role in NR metabolism and homeostasis. Previously, the v-ATPase was also shown to play essential roles in preserving mitochondrial function in yeast (Hughes and Gottschling, 2012). Overexpression of v-ATPase proteins preserved vacuolar acidity and amino acid import, and extended RLS (Hughes and Gottschling, 2012). This RLS was suggested to extend by preserving vacuolar function, allowing proper transport of metabolites between the cytoplasm and vacuole and increasing metabolic flexibility since compromised vacuolar function led to mitochondrial fragmentation and malfunction (Hughes and Gottschling, 2012). Another pathway that links the importance of vacuolar and mitochondrial function to NR/NAD^+ homeostasis and life span extension is amino acid sensing SPS signaling (Tsang et al., 2015). (See the subsection "*Other nutrient sensing pathways and NAD^+ metabolism*") Thus vacuolar and mitochondrial function are also linked and proper metabolism within one compartment may affect the other.

If the vacuole is a major NR production and storage compartment, how is vacuolar NR converted to NAD^+ in the cytosol? We have previously identified Fun26, as a putative NR transporter on the vacuolar membrane (Vickers et al., 2000; Wiederhold et al., 2009; Lu et al., 2011). Recently Boswell-Casteel *et al.* showed that Fun26 is a high affinity transporter with broad range nucleoside and nucleobase substrate affinity, potentially including the nucleoside, NR (Boswell-Casteel et al., 2014). Fun26 is a yeast homolog of the human equilibrative nucleoside transporter (hENT) family, which mediates bi-directional transport of specific nucleosides (Baldwin et al., 2005; Endo et al., 2007; Young et al., 2008). In yeast, the Fun26 protein was reported to have a minor role in NR transport relative to plasma membrane NR transporter, Nrt1 (Belenky et al., 2008). However, deleting *FUN26* causes a similarly significant increase in NR release as that of *nrt1*Δ mutant, and unlike the *nrt1*Δ mutant, the

*fun26*Δ mutant also had increased intracellular NR accumulation (Lu et al., 2011). Therefore the NR balancing function of Fun26 may be key to cytosolic NAD^+ synthesis from the NR pool produced and stored in the vacuole. Currently in higher eukaryotes, cellular NR uptake and transport mechanisms are still unknown, however there are four hENT transporters related to Fun26 (Young et al., 2008). Since yeast Fun26 balances NR pools, perhaps plasma membrane, hENT1 and hENT2 and lysosomal membrane, hENT3, also participate in NR homeostasis and NAD^+ metabolism in humans. Another potential NAD^+ homeostasis compartment is the peroxisome: peroxisomal Nudix hydrolase, Npy1, a NADH pyrophosphatase was shown to produce NMN(H) from NADH (AbdelRaheim et al., 2001) and contribute to NAD^+ homeostasis (Kato and Lin, 2014a). Perhaps Npy1 produces NMN (H) as a storage form of NAD^+ that can be quickly salvaged via NR salvaging (see the subsection "Overview of NAD^+ biosynthesis"). Future studies on novel NAD^+ homeostasis factors will further our understanding of how compartmentalization regulates NAD^+ metabolism and cell functions.

Signaling pathways that regulate and/or cross-talk with NAD^+ metabolism

We previously introduced the *PHO,* SPS, and PKA pathways and NAD^+ metabolism. In this section we will discuss regulation of or cross-talk with NAD^+ metabolism by these and other pathways (Fig. 3).

The PHO pathway and NAD^+ metabolism

Our previous studies linked the regulation of NAD^+ metabolism and phosphate signaling (Lu et al., 2011). In *S. cerevisiae*, phosphate homeostasis is regulated by the *PHO* pathway, which monitors and responds to changes in Pi availability (Lenburg and O'Shea, 1996; Auesukaree et al., 2004) (see the section "Phosphate sensing pathway"). We previously reported that NR production positively correlates with *PHO* activation (Lu et al., 2011): intracellular NR increased under active *PHO* pathway (*i.e*., low Pi growth media or deletion of *PHO84*), and decreased in cells defective in *PHO* signaling (*i.e*., *pho4*Δ mutant). Increased NR production under high *PHO* activity is mostly due to Pho8, since deletion of *PHO8* gene largely decreases intracellular NR level (Lu et al., 2011). Our studies also demonstrated that uptake of extracellular NMN requires prior conversion to NR by periplasmic Pho5, a *PHO*-regulated phosphatase (Lu and Lin, 2010). The cytosolic nucleotidase, Sdt1, which also produces NR from NMN (Bogan et al., 2009), has been suggested to also be under *PHO* regulation (Xu et al., 2013). Altogether, these studies provide evidence for *PHO* regulation of NR homeostasis and NAD^+ metabolism in yeast. Yet in mammals, the method by which cells uptake NAD^+ precursors remains unclear. Mammalian cells do uptake some forms of NAD^+, since supplementing NAD^+ precursors and intermediates was shown to replenish NAD^+ level and ameliorate defects from abnormal NAD^+ metabolism in mammalian models (Sasaki et al., 2006; Bogan and Brenner, 2008; Ramsey et al., 2008; Imai and Guarente, 2014). In addition, NR has been suggested to circulate in the peripheral bloodstream in mammals (Schmidt-Brauns et al., 2001). Perhaps in mammals, large impermeable NAD^+ precursors are converted to NR and other smaller NAD^+ intermediates prior to cell assimilation. To date, phosphate signaling in mammalian cells also remains unclear; and, perhaps NAD^+ metabolism is also linked to phosphate signaling in higher eukaryotes.

In addition to regulating NAD^+ metabolism, *PHO* pathway perhaps responds to rate of NAD^+ metabolism since decreased intracellular NaMN level may activate *PHO* pathway (Lu et al., 2011) (see the subsection "Additional roles of NAD^+ intermediates and biosynthetic enzymes"). The cross-regulation of *PHO* signaling and NAD^+ metabolism is unclear, but coupling these pathways may be metabolically advantageous in certain conditions. For example, the Pi moiety of various NAD^+ derivatives are potential targets for Pi scavenging during Pi limitation. Moreover, as a key intermediate for NAD^+ biosynthesis, low level NaMN might signal impaired NAD^+ biosynthesis and activate alternative NAD^+ salvage routes. Since NR-mediated NAD^+ synthesis requires Pi (in the form of ATP), coordinated activation of the *PHO* pathway is expected to support NAD^+ synthesis and homeostasis.

Other nutrient sensing pathways and NAD^+ metabolism

In the previous section, we discussed the *PHO* pathway and NAD^+ metabolism. Here we will discuss additional nutrient sensing pathways that may regulate and/or cross-talk with NAD^+ metabolism: the SPS, PKA, TOR and Sch9 pathways. In yeast, mutations that reduce SPS amino acid sensing pathway extend life span via increasing NADH shuttle activity and NR homeostasis, both of which contribute to NAD^+ homeostasis. Components of the mitochondrial malate-pyruvate NADH shuttle play an important role in this life span extension (Tsang et al., 2015) (see the subsection "*Mitochondrial compartmentalization of NAD+(H)* " for NADH shuttle). Expression of the malate-pyruvate NADH shuttle components is increased in a low SPS activity mutant, *ssy5*Δ, and the SPS transcription factor, Stp1, binds to the promoters of shuttle components, *MAE1* and *OAC1*. Moreover, deleting *MAE1* and *OAC1* significantly reduced *ssy5*Δ-induced life span extension. NAD^+ homeostasis of *ssy5*Δ is also enhanced by increasing NR salvaging. Deletion of *SSY5* increases NR levels, which partially restores the NAD^+ pool and life span of the short-lived and NAD^+-depleted *npt1*Δ mutant. The main source of increased NR in *ssy5*Δ cells is Pho8, which is required for full life span extension by *ssy5*Δ. Interestingly, *ssy5*Δ-induced *PHO8* expression is independent of the canonical *PHO* pathway and *PHO8* expression likely

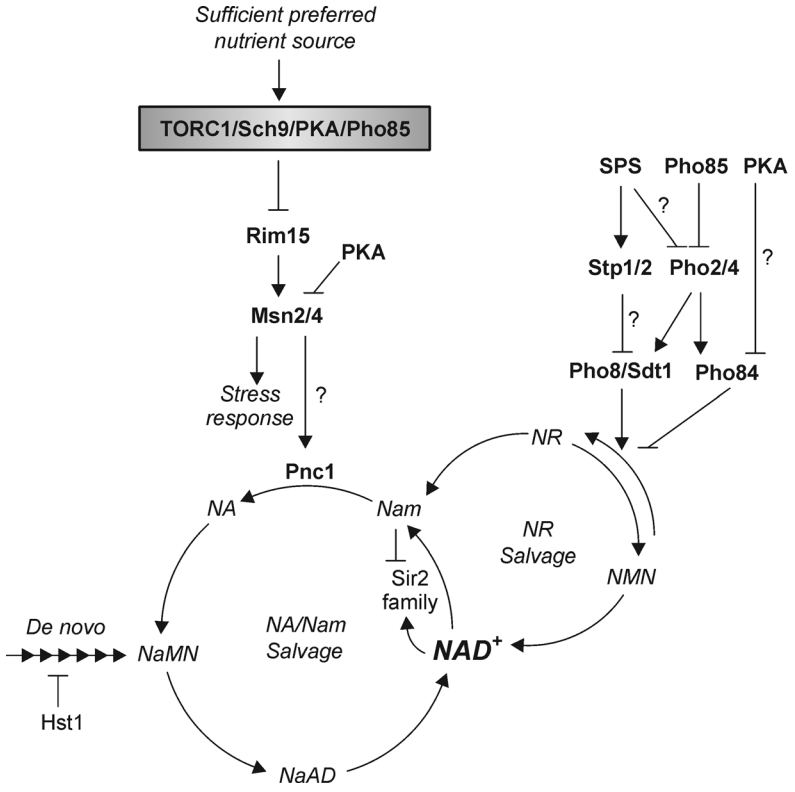

Figure 3 Nutrient sensing pathways that cross-talk with or cross-regulate the NAD^+ biosynthetic pathways. NAD^+ metabolism produces Nam, which is inhibitory to the activity of Sir2 family proteins as NAD^+-dependent protein deacetylases. Also the Sir2 family, Hst1, can repress *de novo* NAD^+ synthesis by silencing the expression of *BNA* genes. TORC1/Sch9/PKA/Pho85 may inhibit NA/NAM salvage via regulating Pnc1. Reduced SPS pathway activity increases *PHO* pathway activity and also increases *PHO8* expression independent of *PHO* pathway, both leading to increased NR salvage activity. Thus active SPS pathway likely represses *PHO* activity. Low levels of phosphate activates *PHO* pathway, leading to concomitant increases in NR salvage activity via phosphatases, Pho8 and Pho5 (not shown), and nucleotidases Sdt1 and Isn1 (not shown). Whereas active Pho85 may activate or inhibit NR salvage activity. When phosphate is plentiful (and *PHO* pathway is suppressed), PKA helps degrade Pho84 phosphate transporter. When *PHO84* is deleted, *PHO* pathway is activated and NR salvage activity increases. TORC1: target of rapamycin complex 1. Sch9: S6 kinase homolog. PKA: glucose-sensing cyclic AMP-protein kinase A pathway. Pho85: cyclin dependent kinase. Rim15: serine/threonine kinase. Msn2/4: zinc finger STRE (stress response element; AGGGG) binding transcription factors. Pnc1: nicotinamide deamidase. SPS: Ssy1-Ptr3-Ssy5 amino acid sensing pathway. Stp1/2: SPS pathway transcription factors. Pho2/4: *PHO* pathway transcription factors. Pho8: vacuolar phosphatase. Sdt1: cytoplasmic nucleotidase. Pho84: plasma membrane phosphate transporter. *de novo*: pathway of NaMN synthesis from tryptophan. *NaMN*: nicotinic acid mononucleotide. *NaAD*: nicotinic acid adenine dinucleotide. NAD^+: nicotinamide adenine dinucleotide. *Nam*: nicotinamide. *NA*: nicotinic acid. *NR*: nicotinamide riboside. *NMN*: nicotinamide mononucleotide. Sir2 Family: Sir2, Hst1, Hst2, Hst3, Hst4.

involves yet-to-be identified transcription factors since Stp1 does not appear to directly bind to *PHO8* promoter (Tsang et al., 2015). Overall, these studies suggest SPS signaling is a regulator of NAD^+ homeostasis. Enhanced NAD^+ homeostasis due to reduced SPS signaling may concomitantly increase mitochondrial and vacuolar function, all contributing to life span extension.

Other pathways that may play a role in NAD^+ metabolism include PKA, Sch9, and TOR. In the section "Phosphate sensing pathway", we discussed the interaction of PKA with PHO signaling. Interestingly, all these pathways may converge on Rim15 to regulate stress response transcription factors, Msn2 and Msn4 (Carroll et al., 2001; Swinnen et al., 2006; Wei et al., 2008). Msn2 and Msn4 have been shown to regulate expression of the NA/NAM salvaging enzyme, *PNC1* (Medvedik et al., 2007). Various mild stresses were observed to increase *PNC1* expression: glucose or amino acid restriction, heat or salt stresses, and mRNA mistranslation (Anderson et al., 2003). Given that mild stress can mediate life span extension (Shama et al., 1998; Kaeberlein et al., 2002; Anderson et al., 2003; Li et al., 2011) Pnc1 may be a key factor connecting stress signals to the regulation of Sir2 (Lamming et al., 2004) and NAD^+ metabolism. Interestingly, Nampt in higher eukaryotes, is also responsive to stresses and may also connect stress to NAD^+ metabolism (Jia et al., 2004; van der Veer et al., 2005). Complex cross-talk between these pathways may allow cells to quickly adapt to environmental changes.

Conclusions and perspectives

Integral to all living organisms, a multitude of nutrient sensing mechanisms allows fine control of cell activity. The budding yeast, *Saccharomyces cerevisiae*, has been used to study nutrient sensing pathways, their cross-talk and cross-regulation. Factors that regulate nutrient sensing can also modulate longevity. Reduced activity of nutrient sensing pathways such as PKA, TOR and Sch9 have been linked to increased life span in yeast and higher eukaryotes (Lin et al., 2000; Fabrizio et al., 2001; Kaeberlein et al., 2005b; Wei et al., 2008; Wang et al., 2009; Longo and Fabrizio, 2012). Recently in yeast, reduced activity of amino acid sensing SPS pathway was also linked to increased life span (Tsang et al., 2015). Metabolic factors such as NAD$^+$ metabolism have also been proposed to play an important role in yeast life span extension.

Pyridine nucleotides are in a myriad of cellular processes and therefore, it is essential to maintain NAD$^+$ homeostasis: aberrant NAD$^+$ metabolism has been implicated in numerous metabolic and age-associated diseases (Imai and Guarente, 2014). Multiple NAD$^+$ synthesis pathways suggest that NAD$^+$ metabolism is dynamic and flexible, ensuring cells can adapt to many environmental changes. And due to this complex nature, factors regulating NAD$^+$ metabolism and homeostasis are not completely understood, although recent studies have identified novel regulators of NAD$^+$ homeostasis. For example, the mitochondria and vacuole were shown to play important roles in the biosynthesis and metabolism of pyridine nucleotides such as NAD$^+$(H) and NADP$^+$(H) (Sies 1982; Bakker et al., 2001; Kato and Lin, 2014a; Lewis et al., 2014). And, generally compartmentalization may be important for cellular control: NAD$^+$ intermediates can be signaling molecules, and high intracellular concentrations can inhibit certain cellular processes. Recent studies support that compartmentalization of pyridine metabolites is pivotal for regulating NAD$^+$ homeostasis and balancing their concentrations is critical for the regulation of many cellular processes. Future studies to understand the multiple roles of NAD$^+$, NAD$^+$ intermediates, as well as novel factors regulating these processes are highly anticipated.

To maintain adequate levels of pyridine nucleotides, changes in NAD$^+$ precursor levels must be sensed. NAD$^+$ metabolism is likely tightly regulated in concert with other nutrient sensing pathways. Recently, NAD$^+$ metabolism was linked to the *PHO* and SPS pathways in yeast. *PHO* pathway activation increases NR production and mobilization (Lu et al., 2011). To extend life span, reduced SPS signaling required increased NAD$^+$ and NR homeostasis (Tsang et al., 2015). Also, cross-regulation of *PHO*, PKA, TOR and Sch9 pathways has been reported to potentially affect NAD$^+$ metabolism (Kato and Lin, 2014a); however, detailed mechanisms remain unclear. Most likely, the complex interplay between nutrient signaling pathways confers essential metabolic flexibility in different growth conditions. Future studies to identify additional signaling factors that regulate cross-talk between NAD$^+$ homeostasis, longevity regulating, and nutrient signaling pathways are highly anticipated. Overall, these studies contribute to our understanding of how nutrient signaling pathways cross-talk with and regulate NAD$^+$ homeostasis pathways, and may also provide further insight into the molecular mechanisms of diseases related to defects in NAD$^+$ metabolism.

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Compliance with ethics guidelines

Felicia TSANG, Su-Ju LIN declare that they have no conflict of interest. This article does not contain any studies with human or animal subjects performed by any of the authors.

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