Cooperation between subcellular NAD pools in the maintenance of NAD-dependent processes

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Thesis for the Degree of Philosophiae Doctor (PhD)
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Scientific environment

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Ingvill Tolås, August 2018

Abstract

Nicotinamide adenine dinucleotide (NAD) is needed as a cofactor in several processes throughout the cell, and its availability is decisive for the activity of many NAD-dependent enzymes. Owing to the membrane barriers in the compartmentalized eukaryotic cell, NAD cannot move freely. Understanding how subcellular NAD pools are established and maintained, as well as how they communicate with each other, is therefore crucial to understanding how NAD dependent processes are regulated in the eukaryotic cell.

In this study we aimed at determining the role of the hitherto uncharacterized peroxisomal NAD pool. Moreover, we addressed experimentally the question as to how subcellular NAD pools communicate and cooperate upon altered NAD homeostasis. To do so we studied the consequences of excessive NAD^+ consumption in the peroxisomes, cytosol or mitochondria using cell lines constitutively expressing the catalytic domain of PARP1 (PARP1cd) targeted to these organelles. The activity of the constructs was confirmed by PAR detection and a 30-40% decline in cellular NAD^+ content. The excessive NAD^+ consumption in peroxisomes did not severely affect the cellular phenotype, and peroxisomal processes were largely maintained. In line with this, upregulated expression of solute carrier SLC25A17 in these cells suggested that import of NAD^+ into peroxisomes was increased to compensate for the excessive consumption. Interestingly, acetylation state of sirtuin targets in various compartments appeared unaltered. Thus, increased import of NAD^+ into peroxisomes seemingly occurred without affecting NAD^+-dependent deacetylation in other organelles. This is likely facilitated by the observed adjustment of the expression of NAD biosynthetic enzymes, NMNATs, and sirtuins in these cells.

Likewise, excessive NAD^+ consumption in the cytosol, and to a lesser extent, mitochondria, was also accompanied by altered expression of NMNATs and sirtuins combined with maintained acetylation state. This indicated that increased local NAD biosynthesis together with altered flux through sirtuins can maintain NAD^+-dependent deacetylation despite significantly lowered cellular NAD^+ content. In sum,

our findings suggest that the maintenance of the peroxisomal pool might be more important than previously assumed. Moreover, we propose the existence of an inter-organellar compensatory response capable of shifting the distribution of NAD within the cells upon compartment specific increase in NAD^+ consumption. Maintenance of NAD^+-dependent signaling in the affected or donating organelle(s) is proposed to occur via altered expression of local NAD producers and consumers.

List of publications

Paper I: VanLinden, M.R.*, Tolås, I.*, Høyland, L.E.,Pettersen, I.N.K., Niere, M., Dölle, C., Mjøs, A.M., Tronstad, K.J., Ziegler, M. Role of peroxisomal NAD in the maintenance of cellular functions.

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Manuscript

Paper II: Tolås, I., VanLinden, M.R., Sverkeli, L.J., Ziegler, M. Compartment specific NAD^+ depletion reveals compensatory communication between NAD pools.

Manuscript

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Selected abbreviations

Nam	Nicotinamide
NA	Nicotinic acid
QA	Quinolinic acid
N(A)R	Nicotinamide (nicotinic acid) riboside
N(A)MN	Nicotinamide (nicotinic acid) mononucleotide
N(A)AD(P)	Nicotinamide (nicotinic acid) adenine dinucleotide (phosphate)
Nam(NA)PRT	Nicotinamide (nicotinic acid) phosphoribosyltransferase
QAPRT	Quinolinic acid phosphoribosyltransferase
NRK	Nicotinamide riboside kinase
NMNAT	Nicotinamide mononucleotide adenylyltransferase
NADS	NAD synthase
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
PRPP	Phosphoribosyl pyrophosphate
(c)ADPr	(cyclic)ADP ribose
OAADPr	2’’- or 3’’-O-acetyl-ADP-ribose
ART	ADP-ribosyltransferase
PARP1 (cd)	Poly-ADP-ribose polymerase 1 (catalytic domain)
PAR	Poly-ADP-ribose
PARG	Poly-ADP-ribose glycohydrolases
TARG1	Terminal ADP-ribose glycohydrolase 1
ARH3	ADP-ribosylhydrolase 3
MD1/MD2	Macrodomain 1 / macrodomain 2
SIRT	Sirtuin
KAT	Lysine acetyltransferase
KDAC	Lysine deacetylase
CR	Calorie restriction
MnSOD2	Manganese superoxide dismutase 2

ROS	Reactive oxygen species
VLCFA	Very long chained fatty acids
LC-MS	Liquid chromatography-mass spectrometry
SLC25A17	Solute carrier family 25 member 17 (also named PMP34)
TCA	Tricarboxylic acid
H3K9	Histone 3 Lysine 9
(Acetyl)-CoA	(Acetyl)-Coenzyme A
PXMP2	Peroxisomal membrane protein 2
PTM	Post translational modification

1. Introduction

Enzymes are important macromolecules which act as catalysts for chemical reactions. Without enzymes, most of the vital biochemical reactions life depends on would not occur in time scales or at temperatures/pressures conducive to life. Cells contain thousands of different enzymes, and tight regulation of their activities determines which of the many possible chemical reactions actually take place within the cell at any given time. Remarkably, most enzymes do not catalyze reactions on their own, but require an additional chemical group, a cofactor, to perform their function. Cofactors can be metal-ions or organic molecules derived from vitamins, and the accessibility of enzymes to their cofactors represents an important layer in the regulation of their activity.

One cofactor is nicotinamide adenine dinucleotide (NAD), which is biosynthetically converted from vitamin B3. NAD can exist in an oxidized (NAD^+) and reduced state (NADH). NAD^+ can be reduced to NADH via a hydride transfer, and then re-oxidized back to NAD^+. As such, it functions as an electron carrier and is used as a co-enzyme by dehydrogenases in many metabolic redox reactions for example in the tricarboxylic acid (TCA) cycle, β-oxidation of fatty acids and amino acid metabolism. In addition, NAD^+ can also be used as a (co)substrate in deacetylation by sirtuins and in mono- or poly-ADP-ribosylation, as well as for the synthesis of second messengers important in calcium signaling. Accordingly, NAD^+ is involved in a wide variety of enzymatic activities, and the regulation of its availability is fundamental to the functionality of the cell.

1.1 NAD and NAD biosynthesis

NAD^+ is a dinucleotide consisting of the two mononucleotides adenosine monophosphate (AMP) and nicotinamide mononucleotide (NMN) joined by their phosphate groups (Fig 1). When used as a (co)substrate NAD^+ is cleaved at the N-glycosidic bond between the nicotinamide (Nam) moiety and ribose on NMN, resulting in the formation of adenosine diphosphate ribose (ADPr) and free Nam. Whereas oxidation and reduction of NAD do not lead to its degradation, signaling reactions *de facto* lower NAD availability and therefore, continuous biosynthesis of the dinucleotide is required. Given the ubiquitous importance of NAD, redundancies have evolved to ensure the maintenance of cellular levels. To that end, several pathways lead to its biosynthesis, differing in precursor used and enzymes involved. Each of these paths starts with the generation of NMN, or its acidic equivalent, nicotinic acid mononucleotide (NAMN) (collectively pyridine mononucleotides) (Fig 2).

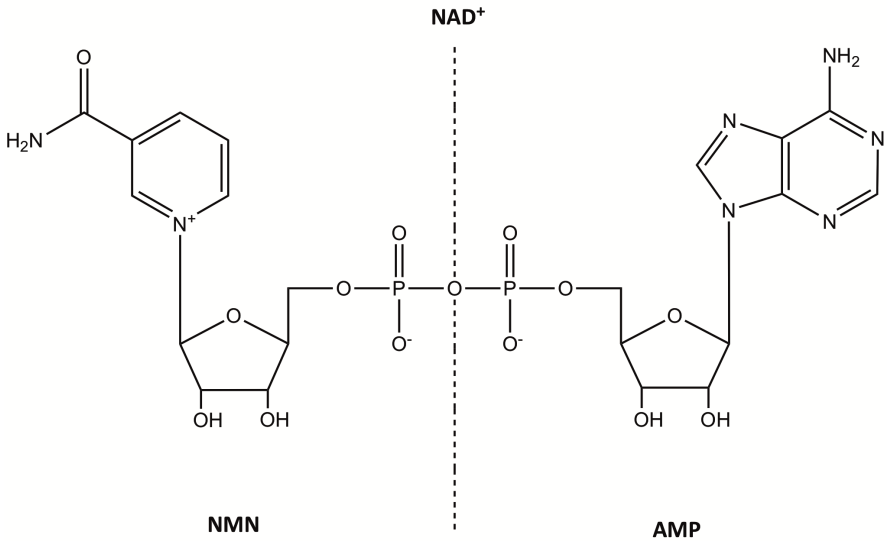

Figure 1. Structure of NAD^+. Nicotinamide adenine dinucleotide (NAD) consists of two mononucleotides; nicotinamide mononucleotide (NMN) and adenine monophosphate (AMP) (indicated by dotted line). The two mononucleotides are joined together via a phosphodiester bond.

Mammalian *de novo* synthesis of NAD starts with the generation of quinolinic acid (QA) from tryptophan through the kynurenine pathway. QA is the substrate for quinolinic acid phosphoribosyltransferase (QAPRT), which catalyzes the conversion of QA to NAMN (Nishizuka & Hayaishi 1963a; Nishizuka & Hayaishi 1963b) using phosphoribosyl pyrophosphate (PRPP) as a co-substrate. Whereas the *de novo* synthesis of NAD^+ relies on the continuous supply of an essential amino acid, the salvage pathway recycles Nam resulting from NAD^+ degrading activities by enzymes like ARTs, sirtuins and glycohydrolases such as CD38/157 (Fig 2). Nam is converted to nicotinamide mononucleotide (NMN) by nicotinamide phosphoribosyltransferase (NamPRT), the rate-limiting enzyme of NAD biosynthesis, which transfers a phosphoribosyl unit from PRPP onto Nam. The affinity of NamPRT towards Nam is remarkably high with a Km of around 5 nM (Burgos & Schramm, 2008), highlighting the importance of utilizing cleaved Nam for resynthesizing NAD. The acidic form of Nam, nicotinic acid (NA), can also be used as a precursor in NAD^+ biosynthesis in what is known as the Preiss-Handler pathway. NA is converted to NAMN by nicotinic acid phosphoribosyltransferase (NAPRT) which, like NamPRT, uses PRPP as a co-substrate (Preiss & Handler 1958a; Preiss & Handler 1958b). Another route to the formation of the pyridine mononucleotides occurs from the ribosylated versions of Nam and NA, nicotinamide riboside (NR) and nicotinic acid riboside (NAR) (Bieganowski & Brenner, 2004) (Fig 2). They are phosphorylated by nicotinamide ribose kinases (NRKs) to NMN and NAMN, respectively. In these reactions, ATP serves as a phosphate donor and ADP is released as a by-product of the reaction. As an alternative to being phosphorylated to NMN, NR can also be cleaved, resulting in Nam which can enter into the salvage pathway (Rowen & Kornberg 1951). Following the generation of the mononucleotide, nicotinamide mononucleotide adenylyltransferases (NMNATs) catalyze the condensation of NAMN or NMN with the adenylyl moiety from ATP, converting the mononucleotides to their respective dinucleotides, nicotinic acid dinucleotide (NAAD) and NAD. The formation of NAD from NAAD requires its amidation, an ATP dependent reaction catalyzed by NAD synthase (NADS) (Fig 2). NAD can further be phosphorylated to NAD phosphate (NADP) by NAD kinase (NADK) in an ATP dependent manner.

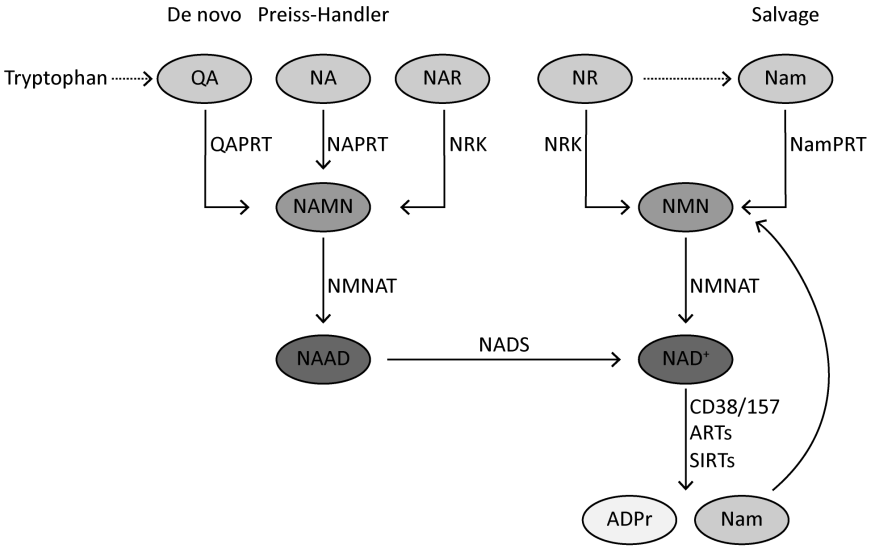

Figure 2. Biosynthesis of NAD$^+$. Biosynthesis of NAD is achieved via either the *de novo*, Preiss-Handler or salvage pathways. NAD$^+$ consuming enzymes degrade NAD$^+$ and cleave it into nicotinamide (Nam) and adenosine diphosphate ribose (ADPr). In the salvage pathway Nam is recycled and converted into nicotinamide mononucleotide (NMN) by nicotinamide phosphoribosyltransferase (NamPRT). In the *de novo* pathway, quinolinic acid (QA) generated from Tryptophan is converted to nicotinic acid mononucleotide (NAMN) by quinolinic acid phosphoribosyltransferase (QAPRT) (Nishizuka & Hayaishi 1963a; Nishizuka & Hayaishi 1963b), while in the Preiss-Handler pathway nicotinic acid (NA) is converted to NAMN by nicotinic acid phosphoribosyltransferase (NAPRT) (Preiss & Handler 1958a; Preiss & Handler 1958b). The ribosylated versions of Nam and NA, nicotinamide riboside (NR) and nicotinic acid riboside (NAR) can be converted to their respective mononucleotides by nicotinamide ribose kinases (NRKs), thus making them precursors for the mononucleotides (Bieganowski & Brenner, 2004). Further, NR can be cleaved into Nam, and thereby enter the salvage pathway (Rowen & Kornberg 1951). Both NAMN and NMN are converted to their respective dinucleotides by nicotinamide mononucleotide adenylyltransferases (NMNATs), creating nicotinic acid adenine dinucleotide (NAAD) and NAD, respectively. NAAD can be converted to NAD by NAD synthase.

1.2 NAD^+ in signalling

While the importance of NAD as a redox carrier has long been known, the new and expanding field of NAD metabolism has arisen from the discovery that NAD^+ and its derivatives can also be used as co-substrates in several signaling reactions (Houtkooper *et al*, 2010; Berthelier *et al*, 1998; Chambon *et al*, 1963; Kirchberger & Guse, 2013; Frye, 1999; Grube & Burkle, 1992).

1.2.1 Calcium mobilization

Molecules derived from NAD and NADP are well established key elements of calcium signaling, mediated by the activity of CD38 and its functional homologue CD157 (Guse, 2015). These ectoenzymes possess both NADase and cyclase activities and utilize NAD^+ to generate ADPr and cyclic ADPr (cADPr) by cleaving the N-glycosidic bond (Howard *et al*, 1993). In order to form cADPr, CD38/157 subsequently catalyze the formation of a glycosidic bond between C1 formerly attached to the Nam and position 1 of the adenine ring (Graeff *et al*, 2009). cADPr is known to stimulate release of calcium from ryanodine-sensitive intracellular stores, namely the endoplasmic reticulum, while ADPr can activate the plasma membrane cation channel TRPM2 and thereby trigger calcium entry from the extracellular space (Lee, 2012; Gasser *et al*, 2006). Under acidic conditions, CD38/157 can exchange the Nam moiety of NADP for NA, creating nicotinic acid adenine dinucleotide phosphate (NAADP). Like the other NAD derived molecules generated by the activity of CD38/157, NAADP also functions as a calcium messenger by activating two-pore channels found in endolysosomes (Lee, 2012; Aarhus *et al*, 1995). Calcium signaling impacts nearly every aspect of cellular life and the release of Ca^{2+} has roles in excitability, exocytosis, motility, apoptosis and transcription. As such, the role of NAD emphasizes its importance in cellular life.

1.2.2 ADP-ribosylation

ADP-ribosylation is a post-translational modification (PTM) where one (mono-ADP-ribosylation) or several (poly-ADP-ribosylation) ADPr units derived from NAD are transferred onto specific amino acids of acceptor proteins. During poly-ADP-ribosylation, the first ADPr unit transferred onto the acceptor protein is attached via an ester bond and then becomes an acceptor site for subsequent ADPr. The ADPr moieties are linked through an O-glycosidic bond, and further elongation results in polymers which are either linear or branched structures of varied length (Fig 3) (Ritter *et al*, 2003; Ruf *et al*, 1998).

ADP-ribosylation is catalyzed by two groups of enzymes; clostridial toxin like ADP-ribosyltransferases (ARTC) and diphtheria toxin like ADP-ribosyltransferases (ARTD). Members of the ARTC family are ectoenzymes, which catalyze mono-ADP-ribosylation of surface receptors as well as secreted and membrane proteins, while the ARTD family consists of both mono- and poly-ADP ribosyltransferases which act inside the cell (Hottiger *et al*, 2010). The most prominent member of the ARTD family is ARTD1, better known as poly-ADP-ribose polymerase 1 (PARP1). This nuclear localized protein consists of several distinct functional domains including a DNA binding domain, an automodification domain and a catalytic domain. PARP1 activity is induced by DNA damage as detected by its DNA binding domain (Langelier *et al,* 2012). Upon activation, PARP1 modifies histones H1 and H2B near the DNA damage, resulting in loosened chromatin structure and the recruitment DNA repair proteins like XRCC1 and DNA polymerase β, with high affinity for poly-ADP-ribose (PAR). PARP1 thereby functions as a sensor for DNA nicks. Activation of PARP1 also leads to its automodification, resulting in its displacement from the DNA, and subsequent inactivation (Fouquerel & Sobol, 2014). Although less active, PARP2 (ARTD2), also localizes to the nucleus and seems to facilitate DNA repair, and cells require the activity of at least one of these PARPs for survival (de Murcia *et al*, 1997; Ménissier de Murcia *et al*, 2003). Additionally there are several other PARP isoforms which localize to the cytosol (Vyas *et al*, 2013).

Like many other PTMs involved in signaling events, ADP-ribosylation is a reversible modification, and as such the PAR is also subject to degradation by enzymes known as poly-ADP-ribose glycohydrolases (PARG) (Fig 3). PARG hydrolyzes the glycosidic bonds between ADPr units, thereby generating free ADPr. There are several PARG isoforms in mammalian cells, which result from alternative splicing events of a transcript encoded by a single gene. Of them only one isoform localizes to the nucleus, whereas cytosolic isoforms account for most of the total cellular PARG activity (Meyer-Ficca *et al*, 2004). In addition to the nuclear and cytosolic isoforms, two smaller isoforms resulting from alternative splicing and alternative translation initiation were predicted localize to the mitochondria and proposed to carry out PARG activity in this organelle (Meyer *et al*, 2007). A later study revealed that while one of these isoforms indeed was mitochondrial, it did not possess PARG activity. Moreover it was found that the only enzyme with PAR degrading activity in mitochondria is ADP-ribosylhydrolase 3 (ARH3) (Niere *et al*, 2012). While hydrolysis of glycosidic bonds between ADPr units is largely catalyzed by PARG isoforms (Bonicalzi *et al*, 2005; Slade *et al*, 2011; Barkauskaite *et al*, 2013), the removal of the terminal ADP-ribose moiety is performed by terminal ADP-ribose glycohydrolase 1 (TARG1), ARH3, MacroDomain 1 or MacroDomain 2, depending on the compartment and the amino acid that is subject to modification (Fig 3) (Niere *et al*, 2012; Jankevicius *et al*, 2013; Rosenthal *et al*, 2013; Sharifi *et al*, 2013; Rack *et al*, 2016).

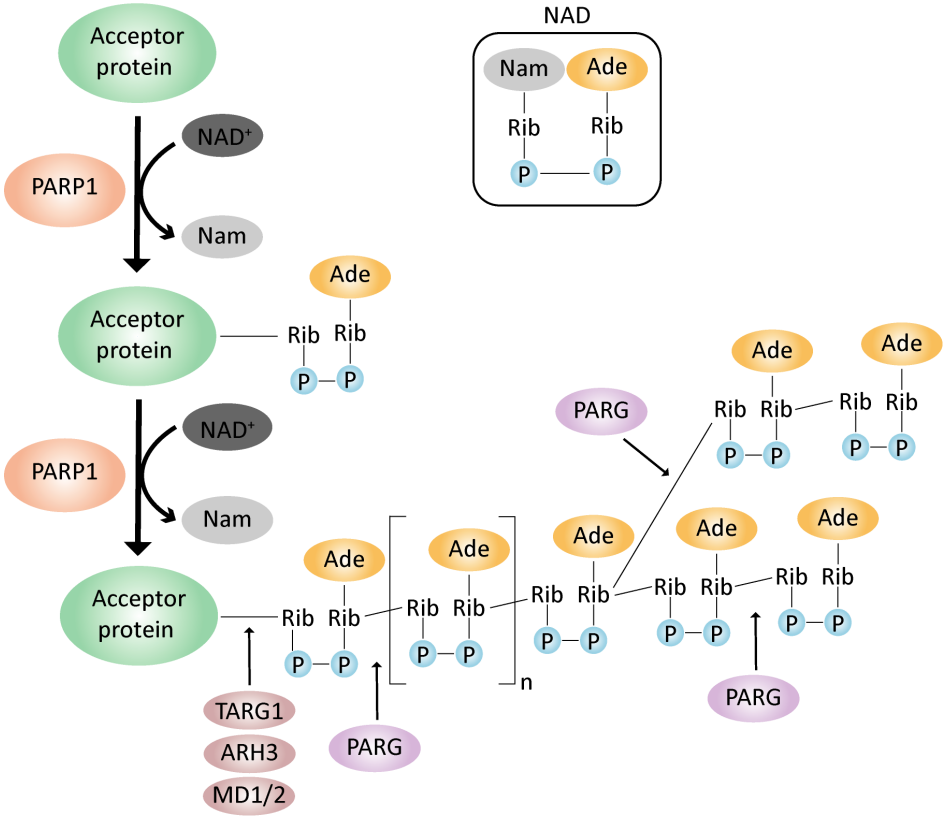

Figure 3. Schematic illustration of poly-ADP-ribosylation of acceptor protein by poly-ADP-ribose polymerase 1 (PARP1). PARP1 catalyzes the hydrolysis of oxidized nicotinamide adenine dinucleotide (NAD^+), releasing nicotinamide (Nam) and ADP ribose (ADPr). The ADPr moiety is subsequently transferred onto acceptor proteins via an ester bond between the initial ADPr and an amino acid acceptor. Following the attachment of the initial ADPr moiety, polymer elongation results from the formation of O-glycosidic bonds between riboses of two successive ADPr units (n is the number of ADPr units). Polymers are also branched, a process achieved via the same chemical reaction as elongation. The first branching occurs after the attachment of about 20 ADPr units. Subsequently it occurs at regular intervals with a distance of 40-50 ADPr units. Poly-ADP ribosylation is a reversible reaction, and the glycosidic bond between ADPr units can be broken by the activity of poly-ADP-ribose glycohydrolases (PARG). Breaking the ester bond between the acceptor protein and the initial ADPr unit requires the activity of terminal ADP-ribose glycohydrolase (TARG1), ADP-ribosylhydrolase 3 (ARH3) or MacroDomain 1 or 2 (MD1/2). NAD: boxed and in dark grey; Nicotinamide: Nam in light grey; Adenine: Ade in orange; ribose: Rib; Phosphate groups: P in blue. (Adapted from Schreiber *et al*, 2006 and VanLinden 2015)

1.2.3 Lysine deacetylation by sirtuins

NAD^+ is also important in the regulation of another ubiquitous PTM, namely the reversible acetylation of ε-aminogroup of lysine-residues. The best-characterized effect of this modification is transcriptional regulation via alterations in chromatin structure. N-ε-lysine acetylation on histone tails neutralizes the positive charge of the lysine, thereby preventing its tight interaction with negatively charged DNA. This alters chromatin architecture and consequently gene transcription (Allfrey *et al*, 1964; Norton *et al*, 1990; Morales & Richard-Foy, 2000; Norton *et al*, 1989; Gansen *et al*, 2015). At the time of its discovery, lysine-acetylation was believed to be histone-specific (Allfrey *et al*, 1964). However, acetylation can also occur on non-histone proteins, where it can regulate catalytic activity, induce conformational changes, compete with other lysine modifications and influence stability (Caron *et al*, 2005; Glozak *et al*, 2005; Arif *et al*, 2007; Tao *et al*, 2010; Guan & Xiong, 2011). Moreover, protein acetylation is not confined to the nucleus. Many mitochondrial and cytosolic proteins have emerged as major acetylation targets (Kim *et al*, 2006; Sadoul *et al*, 2011), and the global importance of acetylation is highlighted by the fact that over 3000 distinct protein acetylation sites have been identified (Choudhary *et al*, 2009). Acetylation state is regulated by two opposing reactions; acetylation and deacetylation (Fig 4). On the one hand, lysine acetyltransferases (KATs) transfer the acetyl group from acetyl coenzyme A (acetyl-CoA) onto the acceptor protein (Berndsen & Denu, 2008; Marmorstein & Roth, 2001). On the other hand, deacetylation is done by two major groups of deacetylases: Zn^{2+} dependent deacetylases (KDACs) (Haberland *et al*, 2009) and a family of NAD^+-dependent deacetylases called sirtuins (Finkel *et al*, 2009; Frye, 1999; Landry *et al*, 2000; Imai *et al*, 2000).

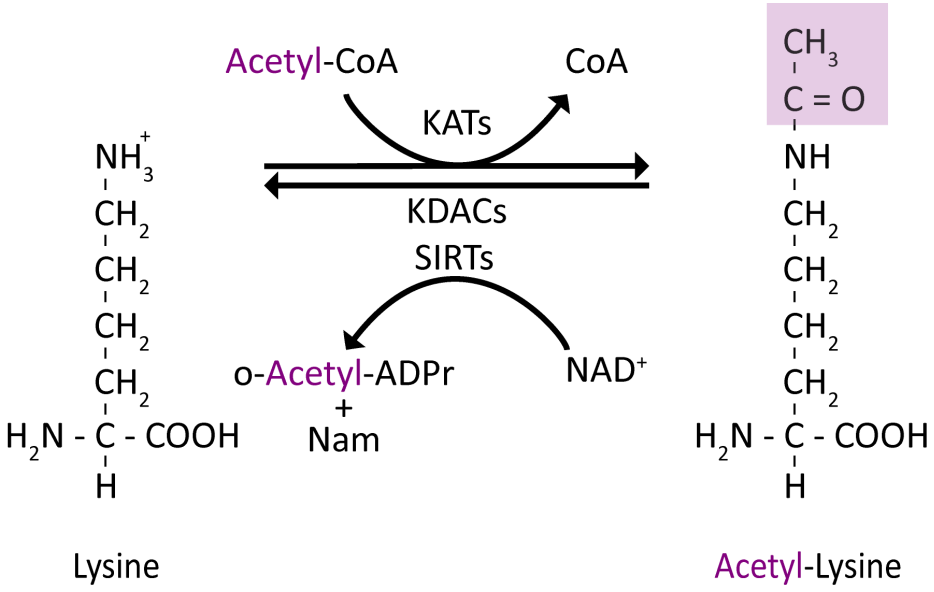

Figure 4. Acetylation and deacetylation of the ε-aminogroup of lysine residues. Lysine acetyltransferases (KATs) transfer the acetyl moiety from acetyl coenzyme A (acetyl-CoA) onto the ε-aminogroup of lysine residues. The acetyl group can be removed by classical lysine deacetylases (KDACs) and NAD^+ dependent sirtuins (SIRTs), which cleave NAD^+ into adenosine diphosphate ribose (ADPr) and nicotinamide (Nam), and then transfer the acetyl moiety from the acetylated lysine to the ADPr, generating o-acetyl-ADPr. **(**Adapted from Kim G-W *et al*, 2010)

In mammals, 7 members of the sirtuin family (SIRT1-7) have been identified (Finkel *et al*, 2009; Frye, 1999). They are expressed in all tissues but vary in their subcellular localization and activity. SIRT1-3 are the predominant isoforms with regards to deacetylase activity. SIRT4 and 6 are able to catalyze mono-ADP-ribosylation, SIRT5 catalyzes demalonylation, desuccinylation and deglutarylation, while SIRT6 can catalyze demyristoylation and depalmitoylation. SIRT7 functions as a deacetylase, but its activity is restricted to the nucleolus (Dali-Youcef *et al*, 2007). In their capacity as deacetylases, sirtuins act upon target lysine residues by coupling deacetylation to NAD^+ hydrolysis, cleaving it into Nam and ADPr and transferring the acetyl group onto the ADPr moiety, creating O-acetyl-ADP-ribose (OAADPr) (Feldman *et al*, 2012). Sirtuins are subjected to product inhibition and can be inhibited by high concentrations of Nam (Bitterman *et al*, 2002).

Among the many processes regulated by acetylation and sirtuin activity, aging has gathered particular attention. During aging, NAD^+ degradation exceeds the biosynthesis and NAD levels decline (Schultz & Sinclair, 2016; Camacho-Pereira *et al*, 2016). The lowered NAD^+ availability and consequent decrease in sirtuin activity leads to alteration in protein acetylation. Indeed, a general increase in histone acetylation leading to a loss of tight gene regulation (De Cecco *et al*, 2013; Li *et al*, 2013; Peleg *et al*, 2010) and an overall rise in mitochondrial acetylation leading to mitochondrial dysfunction, are two of the hallmarks of aging (Schwer *et al*, 2006; Hallows *et al*, 2006; Kim *et al*, 2006; Ozden *et al*, 2011; Sun *et al*, 2016). On the other hand, calorie restriction (CR), the gold standard for inducing increased longevity (McCay *et al*, 1935; Fontana *et al*, 2010), leads to upregulation of NAD^+ biosynthesis, while at the same time decreasing levels of the sirtuin inhibitor Nam (Song *et al*, 2014; Yang *et al*, 2007; Anderson *et al*, 2003). The sum of this is increased sirtuin activity, which is required for the beneficial effects of CR (Chen *et al*, 2005; Lin *et al*, 2000; Cohen *et al*, 2004). Additionally, pharmacological activation of sirtuins mimic benefits from calorie restriction (Wood *et al*, 2004; Mouchiroud *et al*, 2013; Felici *et al*, 2015; Dellinger *et al*, 2017; Rajman *et al*, 2018).

1.3 Subcellular compartmentalization of NAD dependent processes

A fundamental feature of the eukaryotic cell is its organization into distinct subcellular compartments separated by selectively permeable membranes. Each compartment has a specific purpose that contributes to the function of the cell as a whole, and the compartmentalization facilitates the different functions by allowing distinct chemical environments. Several of the compartment specific functions depend on the presence of NAD, either as a redox carrier or as a (co)substrate in signaling reactions. NAD is therefore needed throughout the cell, and distinct pools of NAD have been identified in the nucleus/cytosol, mitochondria and peroxisomes. NAD has also been detected in endoplasmic reticulum and the Golgi-complex, but very little is known about its role in these organelles (Fig 5) (Dölle *et al*, 2010).

Examples of important NAD dependent processes in the nucleus include the detection of DNA damage and subsequent recruitment DNA repair proteins by PARP1 (Langelier *et al*, 2012), and regulation of chromatin structure via histone deacetylation by the nuclear sirtuin isoform, SIRT1 (Zhang & Kraus, 2010). In the cytosol, NAD has a role in glycolysis, where it functions as a redox carrier for glyceraldehyde-3-phosphate dehydrogenase. Other cytosolic NAD^+ dependent reactions include deacetylation of phosphenolpyruvate carboxykinase (PEPCK), forkhead box protein O1 (FOXO1) and □-tubulin by SIRT2, a sirtuin isoform known to predominantly localize to the cytosol (Lin *et al*, 2009; Wang *et al*, 2014; Jing *et al*, 2007; Janke & Montagnac, 2017; North *et al*, 2003). The nuclear envelope does not represent a hindrance to the migration of NAD between compartments, suggesting that these pools are interchangeable. However, a recent paper showed that the nucleus and cytosol represent two separate NAD microdomains (Ryu *et al*, 2018).

In mitochondria, NAD is needed as a coenzyme for many of the functions, such as β-oxidation of fatty acids, the TCA cycle and oxidative phosphorylation, and as a co-substrate for the mitochondrial sirtuin isoform, SIRT3. SIRT3 targets include important proteins such as Manganese superoxide dismutase 2 (MnSOD2) involved in ROS scavenging, and long chained acyl CoA dehydrogenase (LCAD) involved in fatty acid oxidation (Tao *et al*, 2010; Bharathi *et al*, 2013). It is worth noting that SIRT4 and SIRT5 also localize to the mitochondria, but neither of these isoforms possess strong deacetylase activity. Reflecting the many roles of NAD in the mitochondria, it has been suggested that these organelles can contain up to 70% of the cellular NAD (Di Lisa 2002).

In peroxisomes, NAD and NADPH are used by several enzyme involved in important functions, such as β-oxidation of aromatic or cyclic compounds, α and ω-oxidation of very long chained fatty acids (VLCFA), synthesis of bile acids, as well as the generation and removal of hydrogen peroxide (Poirier *et al*, 2006; Wanders, 2014; Waterham *et al*, 2016). Most of the NAD-dependent reactions described in peroxisomes are relying on the oxidation and reduction of NAD/NADPH, and therefore do not lead to a net NAD consumption. Specific examples include L/D-bifunctional enzyme involved in β-oxidation, and catalase, the enzyme responsible for the removal of hydrogen peroxide (Rottensteiner & Theodoulou, 2006; Kirkman & Gaetani, 1984; Kirkman *et al*, 1999). In addition, the NAD(P)(H) degrading enzyme NUDT12, has also been proposed to localize to peroxisomes. This enzyme utilizes NAD(H) as a substrate cleaving it into NMN(H) and AMP (AbdelRaheim *et al*, 2003; Carreras-Puigvert *et al,* 2017). These metabolites can then diffuse out of the peroxisomes via pores like PXMP2 (Antonenkov & Hiltunen, 2012). However, the biological role of NUDT12 activity remains poorly understood. Furthermore, a recent study revealed that several peroxisomal proteins are ADP-ribosylated (Bilan *et al*, 2017). Whether this occurs before or after import into the peroxisomes remains unclear, but it could point to a hitherto unknown NAD^+ consuming process in peroxisomes. Despite the evident importance of NAD in this organelle, the peroxisomal NAD pool remains one of the least characterized pools to date.

1.4 Establishment and maintenance of NAD pools

Given the many NAD^+ consuming processes taking place in the various compartments, NAD^+ must be continuously replenished in each of these organelles. Isoforms of all enzymes required for NAD biosynthesis are present in the cytosol and provide NAD for the cytosol and nucleus. NAD generation for these pools is also supported by the activity of NMNAT1, located in the nucleus. However, the selectively permeable membranes surrounding the remaining NAD pools do not permit NAD to diffuse freely. Thus, these organelles require an alternative mechanism for maintaining NAD levels. This could occur either via transport across the membrane or via autonomous NAD biosynthesis (Fig 5).

In plants and yeast, the mitochondrial NAD pool is established and maintained by the activity of transporters, importing NAD across the inner mitochondrial membrane (Todisco *et al*, 2006; Palmieri *et al*, 2009). While it has been suggested that mitochondria from mammalian cells are capable of importing NAD (Davila *et al*, 2018), the identity of a human mitochondrial NAD transporter has yet to be established. Moreover, it is known that humans express a mitochondrial NAD biosynthetic enzyme, namely NMNAT3 (Lau *et al*, 2010; Nikiforov *et al*, 2011; VanLinden *et al*, 2015). This raises the possibility of autonomous NAD^+ biosynthesis in these organelles. This option also poses challenges like the import or synthesis of the NMNAT3 substrate NMN in the mitochondria. Given the jump in complexity from yeast and plants to humans, and the importance of NAD in the mitochondria, it is possible that there is redundancy with regards to the maintenance of this NAD pool. Thus, compartment specific biosynthesis involving NMNAT3 could work in concert with a mitochondrial NAD transporter. For the peroxisomal pool, a previous study found that the peroxisomal solute carrier family 25 member 17 (SLC25A17) is capable of importing of NAD^+ into reconstituted proteoliposomes (Agrimi *et al*, 2012). However, the role of this carrier in the maintenance of peroxisomal NAD levels has not been confirmed in human cells. It has also been proposed that the NAD^+ consuming activity of NUDT12 could function to regulate peroxisomal NAD levels (Abdelraheim *et al*, 2003; Nikiforov *et al*, 2015).

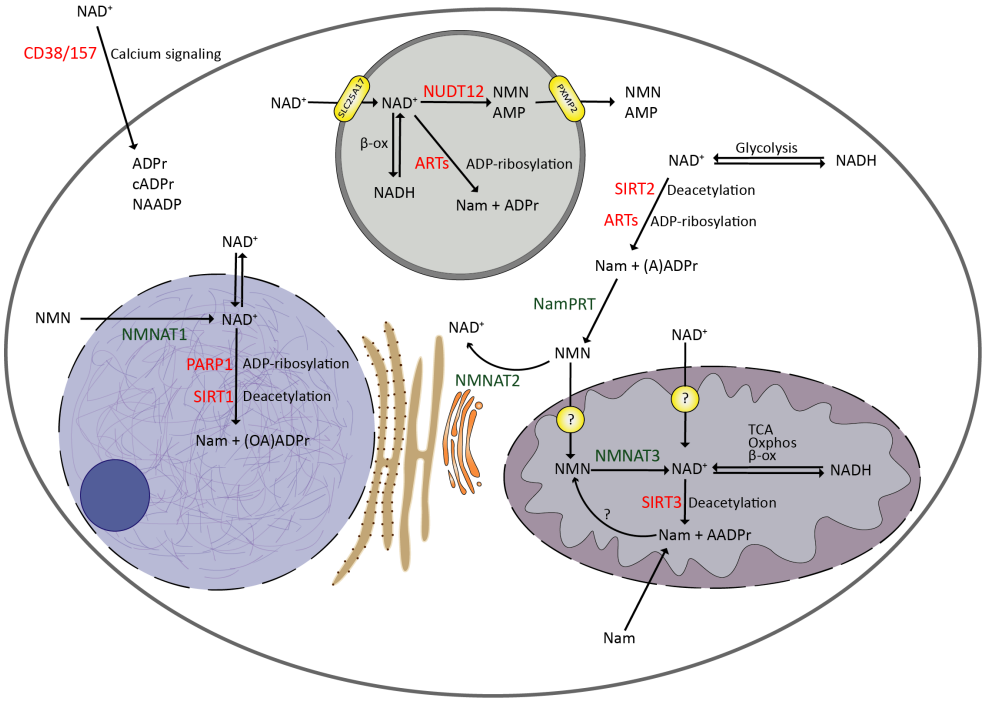

Figure 5. Overview of NAD salvage, NAD shuttling and NAD dependent processes in the nucleus, cytosol, mitochondria and peroxisomes. Extracellular NAD^+can be used to generate calcium signaling molecules ADP ribose (ADPr), cyclic ADP ribose (cADPr) and nicotinic acid adenine dinucleotide phosphate (NAADP) by CD38 or CD157. In the cytosol NAD can be used as a redox carrier in glycolysis or as a cosubstrate by ADP-ribosyltransferases (ARTs) or SIRT2. Nicotinamide (Nam) resulting from these reactions can be converted to nicotinamide mononucleotide (NMN) by nicotinamide phosphoribosyltransferase (NamPRT) and further to NAD by nicotinamide mononucleotide adenylyltransferase 2 (NMNAT2). Cytosolic NAD can also move into the nucleus where it can be utilized by nuclear ADP-ribosyltransferases such as poly-ADP-ribosyl polymerase 1 (PARP1) for ADP-ribosylation, or by SIRT1, for protein deacetylation. NMN from the cytosol can also be converted to NAD by the activity of NMNAT1 inside the nucleus. In peroxisomes, NAD is used as a redox carrier for processes such as □-oxidation. Additionally, ADP-ribosylation of several peroxisomal suggests the presence of an ART activity in this organelle. NUDT12 can cleave NAD^+ into NMN and AMP, metabolites that can diffuse through peroxisomal pore PXMP2. It has been proposed that NAD^+ is transported into peroxisomes by SLC25A17. In mitochondria, NAD is needed as a redox carrier in metabolic processes like the TCA cycle, oxidative phosphorylation and □-oxidation. It is also used for deacetylation by mitochondrial sirtuin isoform SIRT3. The presence of NMNAT3 in mitochondria (VanLinden *et al*, 2015) suggests that there is local NAD biosynthesis from NMN. It has also been shown that mitochondria can import NAD from the cytosol, and the import of NMN cannot be excluded (Davila *et al*, 2018). Whether there is a NamPRT within mitochondria capable of converting Nam generated within the organelle or diffusing from the cytosol into NMN is not known (represented by questionmark). Oxidative phosphorylation: Oxphos; □-oxidation: □-ox

1.5 Communication between the NAD pools

It is well known that communication and cooperation between organelles is vital for the wellbeing of the cell. In line with this there is functional communication and interdependence between organellar NAD-dependent processes. For example, while peroxisomes are responsible for the NAD-dependent β-oxidation of VLCFA, they do not contain the machinery to fully oxidize fatty acids to CO_2. As such, peroxisomes generate a range of chain-shortened acyl-CoAs, while mitochondria are responsible for completing the catabolism. This occurs via mitochondrial β-oxidation and eventually through the TCA cycle, also NAD-dependent processes (Fransen *et al*, 2017). The TCA cycle also completes the catabolism of acetyl-CoA generated from glycolysis, a cytosolic NAD-dependent process.

Owing to dehydrogenase shuttles transporting reducing equivalents across membranes, there is also redox interplay between NAD containing organelles. That is, disturbances in one organelle can affect the redox balance in another (Lismont *et al*, 2015). The malate-aspartate shuttle and the glycerol-3-phosphate shuttle allow NADH generated from glycolysis taking place in the cytosol to be re-oxidized to NAD^+, while NAD^+ inside the mitochondrial matrix is reduced to NADH. The sum is continued glycolysis in the cytosol while NADH in the mitochondria can be used as an electron donor in the electron transport chain, facilitating the production of ATP (Kane, 2014). Furthermore, NADH resulting from redox reactions within the peroxisomes needs to be continuously re-oxidized. In yeast this is achieved via a malate/oxaloacetate or glycerol-3-phosphate dehydrogenase 1(Gpd1p)-dependent shuttle (van Roermund *et al*, 1995; Al-Saryi *et al*, 2017). In higher eukaryotes the exact mechanism for re-oxidation remains unknown, but is ultimately achieved via a dehydrogenase shuttle (Baumgart *et al*, 1996; Visser *et al*, 2007; Antonenkov & Hiltunen, 2012). NADPH is replenished from $NADP^+$ via isocitrate dehydrogenases (Geisbrecht & Gould, 1999).

Despite the functional communication and redox interdependence, it is still unclear whether NAD pools communicate and cooperate with each other with regards to NAD availability. While compartmentalization allows cells to regulate NAD dependent processes by modulating the relative access of NAD dependent enzymes to their co-substrate (Ryu *et al*, 2018), rapid redistribution would allow organelles to respond efficiently to altered NAD demand in a given organelle Although the membranes surrounding the NAD containing organelles prevent NAD from moving freely within a cell, there are several mechanisms in place potentially allowing for communication between pools. On the one hand, dehydrogenase shuttles represent very concrete interplay between the NAD pools, which does not require the movement of the dinucleotide itself. On the other hand, the presence of a proposed NAD^+ carrier in peroxisomes (Agrimi *et al*, 2012) and the ability of mitochondria to import NAD^+ (Davila *et al*, 2018) opens the possibility that communication can occur via actual shifts in NAD^+ distribution. Despite these possible mechanisms, very little is known about how compartments cooperate to maintain NAD homeostasis with regards to distribution between the pools. Importantly, better insight into the regulation of subcellular NAD distribution could have major repercussions for our understanding of how NAD-dependent processes are controlled and maintained.

2. Aims of the thesis

Given the ubiquity of pathways dependent on NAD, the regulation of NAD availability is vital to the wellbeing of cells. An important aspect of this regulation is the compartmentalization of eukaryotic cells. Thus, elucidating how NAD pools are established and maintained, as well as how they communicate and cooperate is key to truly understanding the regulation of NAD dependent processes.

Peroxisomes are vital organelles which function as cellular redox hubs, contribute to ROS metabolism, and are key to energy metabolism. NAD is known to function as a coenzyme in several reactions relevant to these functions, but little is known about the establishment and maintenance of peroxisomal NAD pool, and the role of the peroxisomal NAD pool to overall cellular NAD homeostasis and maintenance of cellular phenotype. To understand the role of peroxisomal NAD, the first part of the study aimed at determining how altered peroxisomal NAD homeostasis affected peroxisomal and cellular functions. Additionally, the presence of NAD in peroxisomes in the absence of local biosynthesis machinery suggests the presence of an NAD^+ carrier. While the carrier protein SLC25A17 has previously been shown to transport NAD^+ into liposomes, this work aimed at confirming the role of this protein in human cells.

Although the activity of NAD dependent enzymes is regulated by NAD availability on a cellular level, it is not known whether and how subcellular NAD pools communicate and cooperate with regards to local NAD availability. Furthermore, the relative importance of subcellular NAD pools and NAD dependent processes within these pools also remains unclear. These questions are fundamental to the understanding of how NAD-dependent processes are regulated and maintained. The second part of this study therefore aimed at determining how organelles respond and communicate in times of altered NAD homeostasis in the various compartments.

3. Summary of results

3.1 Importance and maintenance of the peroxisomal NAD pool

Paper I investigated the role of the peroxisomal NAD pool with regards to regulation of both peroxisomal and non-peroxisomal processes, as well as the establishment and maintenance of this pool. Targeted expression of the catalytic domain of PARP1 (PARP1cd) and PAR formation has previously been used as a tool to study the mitochondrial NAD pool (Niere *et al*, 2008; Nikiforov *et al*, 2011; VanLinden *et al*, 2015, VanLinden *et al,* 2017). Using the same principle, we generated a cell line stably expressing PARP1cd targeted to peroxisomes (293pexPARP1cd), thereby inducing excessive NAD^+ consumption in these organelles. The activity and localization of the PARP1cd construct was confirmed by PAR immunodetection. LC-MS analysis determined that PARP1cd activity resulted in a 40% decline in total NAD^+ content compared to parental 293 cells. Additionally, we found that the polymers were subject to turnover, allowing us to use PAR-detection as a read-out for peroxisomal NAD^+ levels (Fig 3). Despite lowered cellular NAD^+ levels, cellular functions like glycolysis and mitochondrial respiration remained unaltered. Although β-oxidation of VLCFA in peroxisomes depends on NAD as a redox carrier, we found that increased NAD^+ consumption in peroxisomes only led to a small build up of lignoceric acid (C24:0) and behenic acid (C22:0), and a slight decrease in palmitic acid (C16:0) and myristic acid (C14:0). Conversely, increased consumption of mitochondrial NAD^+ had dramatic consequences for the fatty acid profile of the cell. Furthermore, we found that catalase activity and ROS clearance remained nearly unaltered in 293pexPARP1cd cells. In addition, we found that increased NAD^+ consumption in peroxisomes led to upregulated transcription of SLC25A17. Using polymer-formation as a read-out, we found that knocking down SLC25A17 led to lowered NAD^+ availability in peroxisomes. Thus, our data suggest that increased NAD^+ consumption in peroxisomes could, at least in part, be compensated for by upregulated expression of SLC25A17.

3.2 Compensatory communication between subcellular NAD pools.

Manuscript II addressed how altered NAD homeostasis affects subcellular NAD pools, as well as how these pools communicate and cooperate. The selected tools for this were stably transfected cell lines constitutively expressing the catalytic domain of PARP1cd targeted to either the cytosol, peroxisomes or mitochondria. The activity of the constructs was confirmed via immunoblot analysis of PAR and lowered cellular NAD^+ levels as measured by LC-MS analysis. We found that PARP1cd expression in the cytosol and mitochondria resulted in increased ADP levels, a parameter that was unaffected when PARP1cd was targeted to peroxisomes. Sirtuin activity was chosen as a read-out to investigate how compartment specific alterations in NAD^+ consumption affected organellar NAD^+-dependent signaling and availability. Protein acetylation of selected sirtuin targets remained unaltered upon targeted PARP1cd expression in all cell lines. Control cells treated with an inhibitor of NAD-biosynthesis, FK866, showed hyperacetylation of α-tubulin lysine 40 and a hypoacetylation of histones in general, and H3 lysine 9 in particular. By incubating cells in the presence of PARP inhibitor 3-Aminobenzamide and subsequently releasing the inhibition, we were able to observe the effects of acute compartment specific NAD^+ consumption. Sudden NAD^+ consumption also had very little effect on protein acetylation, though H3 lysine 9 was hypoacetylated in cells with PARP1cd targeted to the mitochondria. Furthermore, we looked into the expression of the compartment specific enzymes involved in regulating local availability of NAD^+, namely sirtuin and NMNAT isoforms. We found that increased consumption of NAD^+ in the cytosol led to increased expression of SIRT2 and NMNAT2, the cytosolic isoforms of these enzymes. Upon increased NAD^+ consumption in peroxisomes we observed upregulation of SIRT2, SIRT3, NMNAT1 and NMNAT2, while increased NAD^+ consumption in mitochondria only led to a slight down-regulation of SIRT2.

4. General discussion

Owing to its central role in both metabolic processes and signaling reactions, NAD availability influences essentially every cellular function. The profound impact of modulating NAD levels has been studied on a cellular level, where it was for example shown that NAD depletion through biosynthesis inhibition or excessive NAD^+ consumption can result in major metabolic phenotypes and cell death (Billington *et al*, 2008; Cea *et al*, 2013; Wang *et al*, 2013; Xiao *et al*, 2016). On the other hand, increasing NAD availability protects cells against oxidative stress (Hara *et al*, 2007). Similarly, increasing NAD availability has beneficial consequences on an organismal level, as demonstrated for example in mice with amelioration of metabolic diseases and improved heath span (Barbosa *et al*, 2007; Yoshino *et al*, 2011; Bai *et al*, 2011; Kumar *et al*, 2013; Gomes *et al*, 2013; Mitchell *et al*, 2018; Gulshan *et al*, 2018). The compartmentalization of the human cells allows for an additional level of regulation. Understanding the mechanisms underlying the regulation of each subcellular NAD pools, as well as their role and possible communication will therefore provide further information regarding NAD metabolism.

4.1 The unrecognized importance of the peroxisomal NAD pool

In order to evaluate the function and importance of the peroxisomal NAD pool, we generated a stable cell line (293pexPARP1cd) where peroxisomal NAD^+ consumption is increased by targeted expression of PARP1cd. PARP1cd consumes NAD^+ to generate immunodetectable PAR, and the extent of polymer formation is dependent on NAD^+ availability (Niere *et al*, 2008). Remarkably, expression of PARP1cd in the peroxisomes led to a 40% decrease in cellular NAD^+ levels and resulted in extensive PAR formation similar to that observed upon expression of PARP1cd in the mitochondria. Whereas mitochondria have been reported several times to contain high levels of NAD (Di Lisa *et al*, 2001; Di Lisa 2002; Alano *et al*, 2007), these observations suggest that peroxisomal NAD might contribute more to total cellular NAD levels than previously assumed. To date, most NAD dependent

enzymes in peroxisomes use NAD as a redox carrier, and the only NAD(H) consuming enzyme proposed to localize to peroxisomes is NUDT12 (AbdelRaheim *et al*, 2001; AbdelRaheim *et al*, 2003). These activities would not lead to a high consumption of NAD^+. This begs the question of why peroxisomes would contain substantial amounts of NAD.

Interestingly, a recent study found that important peroxisomal proteins, such as catalase and 3-ketoacyl-CoA thiolase, are subject to ADP-ribosylation (Bilan *et al*, 2017). Whether this modification occurs prior to import or within peroxisomes is unknown, but it does show that NAD^+-dependent signaling could affect peroxisomal proteins. Upon incubation of cells in 3-AB, an inhibitor of PARP activity, we observed that the polymers gradually disappeared. This demonstrated that, regardless of where the modification takes place, PAR is subject to degradation within peroxisomes and the amount of ADP-ribosylation can thereby be regulated locally. Given that several members of the Nudix hydrolase family are capable of degrading PAR (Daniels *et al*, 2015), the peroxisomally located members of this family could seem like possible candidates. However, it has been shown that although NUDT12 is capable of hydrolyzing free ADPr, it is incapable of cleaving PAR (Palazzo *et al*, 2015). Additionally, NUDT7 and NUDT19 only possess acyl-CoA diphosphatase activity (Gasmi & McLennan 2001; Ofman *et al*, 2006). The observed PAR degrading activity could therefore indicate the existence of a peroxisomal PARG or ARH isoform. Although further studies are needed to determine the identity of the enzyme responsible, the PAR degrading activity in peroxisomes provided us with a functional system in which PAR could be used as a read-out for alterations of peroxisomal NAD^+ levels.

To sustain the large amount of PAR formation observed in the 293pexPARP1cd cell, one would expect increased influx of NAD^+ into peroxisomes. In line with this, we found that expression of SLC25A17 was upregulated upon expression of PARP1cd in the peroxisomes. Furthermore, we found that peroxisomal NAD^+ availability is at least partially dependent on the expression of this transporter. Whereas the ability of this carrier to import NAD^+ has previously been described in reconstituted liposomes

(Agrimi *et al*, 2012), the present study reports, for the first time, a physiological role in human cells. While SLC25A17 has been shown to function via a counter exchange mechanism, the *in vivo* counter-substrate for NAD^+ import remains unknown. Thus, further research is required for the full characterization of SLC25A17 in human cells and its role in peroxisomal function. In sum, the strong PAR signal and the increased SLC25A17 expression indicate that cells adapt to increased peroxisomal NAD^+ consumption by adjusting NAD^+ import into these organelles. This could suggest that NAD^+ content in peroxisomes was largely preserved despite increased consumption.

In line with this hypothesis, increased consumption of NAD^+ in peroxisomes led to a small build up of the VLCFAs lignoceric acid (C24:0) and behenic acid (C22:0), and a slight decrease in palmitic acid (C16:0) and myristic acid (C14:0). The small build up of VLCFA indicates a slight impairment of peroxisomal β-oxidation, probably due to a shortage of NAD^+ available for the L/D- bifunctional enzyme. The effect on palmitic acid (C16:0) and myristic acid (C14:0) could reflect the resulting decreased substrate-supply for mitochondrial β-oxidation (Wanders *et al*, 2016; Fransen *et al*, 2017). Though interesting, these effects were minor compared to the effects of similarly altered NAD^+ homeostasis in mitochondria, where NAD^+ depletion was accompanied by a large accumulation of lauric (C12:0) and myristic acid (C14:0). We therefore concluded that peroxisomal β-oxidation was largely maintained despite the increased NAD^+ consumption. It is known that several fatal disorders, such as X-linked adrenoleukodystrophy, Acyl-CoA oxidase deficiency and D-bifunctional protein (DBP) deficiency, are caused by disturbances of peroxisomal β-oxidation and subsequent accumulation of VLCFAs,(Wanders & Waterham, 2006; Ferdinandusse *et al*, 2006; Kemp *et al*, 2012; Wiesinger *et al*, 2013; Abe *et al*, 2014; Wanders, 2014). Maintenance of peroxisomal NAD levels, and subsequently of β-oxidation, is therefore likely to be critical for cellular wellbeing.

ROS metabolism is another important peroxisomal parameter that appeared to be maintained in spite of increased NAD^+ consumption. Since ROS generation is a side-product of peroxisomal metabolism (Schrader & Fahimi, 2006; Del Río & López-Huertas, 2016), this observation is in line with the preserved β-oxidation of VLCFA. Importantly, NAD availability can also be directly linked to ROS generation via its role as a redox carrier to xanthine oxidoreductase (XDH/XO). This enzyme shifts from dehydrogenase to oxidase activity in the absence of NAD^+, resulting in the generation of ROS (Saksela & Raivio, 1996; Nishino *et al*, 2008; Del Río & López-Huertas, 2016). Maintained ROS level is therefore further indication that peroxisomal NAD^+ levels were largely maintained despite increased consumption. While ROS levels remained unaltered, increased NAD^+ consumption led to a small increase in catalase activity. Thus, it is possible that increased NAD^+ consumption resulted in marginally increased ROS generation, but that a slight upturn in catalase activity was sufficient to counteract this effect, thereby preserving ROS levels. Interestingly, increased catalase activity occurred in spite of lowered cellular levels of its cofactor, NADPH (Kirkman & Gaetani, 1984; Kirkman *et al*, 1999). This suggests that local NADPH levels were high enough to sustain catalase activity.

4.2 Communication and cooperation between subcellular NAD pools

The apparent lack of phenotype in a system with increased peroxisomal NAD^+ consumption suggested the presence of mechanisms dedicated to adjusting and maintaining NAD concentrations in various subcellular compartments. To further our understanding of such mechanisms, cells with enhanced NAD^+ consumption in the cytosol, mitochondria or peroxisomes were investigated. Owing to the distribution of sirtuins and their dependence on NAD^+ availability (Cantó *et al*, 2009; Bai *et al*, 2011; Cantó *et al*, 2012; Guan *et al*, 2017; Mouchiroud *et al*, 2013), we reasoned that acetylation state of selected sirtuin targets would reveal how their resident organelle was affected. That is, if the maintenance of one NAD pool occurred at the expense of another, the affected sirtuin target would reveal the NAD^+ donating organelle. Remarkably, while the targeted expression of PARP1cd resulted in a significant decrease of 30-40% in cellular NAD^+ content in all cell lines, it had no apparent effect on the acetylation state of any of the selected sirtuin targets.

In addition to the redistribution of NAD between the organelles, cells might adapt to maintain NAD levels in specific organelles by altering the expression of NAD biosynthetic enzymes. We found that cells responded to increased organellar NAD^+ consumption by altering the transcription of compartmentalized NAD biosynthetic enzyme NMNAT. Whereas these results have yet to be confirmed on the protein level, they indicate a cellular response to NAD^+ consumption. In sum, the changes in NMNAT transcription, together with maintenance of peroxisomal functions and acetylation state of sirtuin targets could suggest that there is a two-fold compensatory response to increased compartmental NAD^+ consumption. That is, NAD is reallocated from various subcellular NAD pools into the targeted compartment, a process accompanied by attempted increased local NAD production in the targeted or donating organelle(s). Notably, the altered expression pattern of NMNAT isoforms was specific depending on the organelle subjected to NAD^+ depletion. This pattern could give an indication of which organelles were involved in each situation. Upon depletion of the cytosolic NAD pool, only NMNAT2 transcription was affected. This

could suggest that, in this case, NAD might not have been reallocated from other pools, and increased local NAD biosynthesis may have sufficed. In line with the suggested importance of maintaining the peroxisomal NAD pool, increased NAD^+ consumption in peroxisomes led to the upregulation of both NMNAT1 and NMNAT2, suggesting that both the nuclear and cytosolic NAD pools were affected, and could be donating NAD to the peroxisomes. Transcription of NMNAT3 was unaffected in all cell lines.

As demonstrated by lowered cellular NAD levels and unaltered NamPRT expression (VanLinden, unpublished observations) there does not appear to be a general increase in NAD biosynthetic capacity in the PARP1cd cell lines. Altered NMNAT transcription could therefore reflect a response in which NAD availability in the resident organelles is maintained by directing NMN away from other subcellular NAD pools, rather than producing more total NAD. This is supported by the observed overall decrease in cellular NAD. In line with this it has been shown that in undifferentiated 3T3-L1 cells, NMN is predominantly used by NMNAT1 to synthesize NAD in the nucleus. Upon differentiation there is an increase in glucose metabolism, and NMNAT2 expression is upregulated to support high cytosolic NAD^+ demands. This in turn lowers the availability of nuclear NMN and NAD synthesis by NMNAT1 (Ryu *et al*, 2018). Whether similar dynamics are present in the PARP1cd cell lines must be experimentally tested, but could represent another layer of communication between the compartments. Thus, increased transcription of NMNAT2 upon cytosolic NAD^+ depletion may be accompanied by a corresponding decrease in nuclear NAD production, partially facilitated by unaltered NMNAT1 transcription and an equivalent shift in the ratio between the isoforms.

Interestingly, preliminary data (vanLinden *et al*, unpublished observations) show that NAD content in isolated mitochondria is lowered in all cell lines, but there is no significant change in the transcription of NMNAT3. Given the demonstrated importance of the mitochondrial NAD pool (Yang *et al*, 2007; Ozden *et al*, 2011), one might assume that cells would attempt to compensate for increased consumption of NAD^+ in mitochondria rather than maintaining other subcellular NAD pools.

However, there are several possible explanations for NMNAT3 remaining unchanged, depending on the understanding of how the mitochondrial NAD pool is established and maintained. Due to the importance and size of the mitochondrial NAD pool (Di Lisa *et al*, 2001; Di Lisa 2002; Alano *et al*, 2007), an intriguing possibility is that mitochondria contain more NAD than their basal requirement. As NAD concentrations fluctuate depending on nutrient intake (Goldberger *et al*, 1915; Aykroyd, 1930; Houtkooper *et al*, 2010), such abundance could serve as a buffer in times of lower nutritional supply of NAD precursors. Assuming that NAD is generated from NMN within the mitochondria (Berger *et al*, 2005; Nikiforov *et al*, 2011; VanLinden *et al*, 2015), it is possible that excess NAD within the organelle allows it to stay out of the competition for available NMN. That is, the mitochondrial pool might contribute to maintenance of NAD in other organelles by not laying claim to available NAD precursor. Moreover, owing to the possible existence of a NMN transporter and hypothesized surplus NAD content in the mitochondria, another exciting possibility is that NMNAT3 consumes NAD to generate NMN for subsequent export out of the mitochondria. The resulting NMN could then be used as a NAD precursor in other organelles. However, if this were the case, one might expect NMNAT3 transcription levels to be upregulated in times of increased NAD^+ consumption in the cytosol or peroxisomes, and lowered upon increased NAD^+ consumption in mitochondria, effects that were not seen. Notably, the mitochondrial Nudix hydrolase NUDT13 is also capable of cleaving NAD, and could fulfill the same role (AbdelRaheim *et al*, 2017).

Importantly, recent studies have called into question the role of NMNAT3 as the sole provider of mitochondrial NAD (Yamamoto *et al*, 2016; Davila *et al*, 2018). While these studies do not eliminate the possibility of a mitochondrial NMN transporter or NMNAT3 as contributing participants, they suggest the existence of an alternate source of mitochondrial NAD, namely an NAD transporter. Considering this possibility, interpretation of the unaltered NMNAT3 transcription would depend on the relative contribution of transport vs autonomous NAD biosynthesis, both under normal conditions and upon altered NAD homeostasis. The existence of such a transporter also opens the possibility that NAD is being exported from the

mitochondria. However, while similar transporters have been characterized in yeast and plants (Todisco *et al*, 2006; Palmieri *et al*, 2009), the proposed human homologue remains unidentified. As such, whether and how the expression of the proposed mitochondrial NAD transporter is affected by a shift in NAD^+ consumption remains to be elucidated.

The hypothesis that mitochondria contain a reservoir of surplus NAD could also explain how pexPARP1cd cells cope with a 40% decrease in cellular NAD^+ levels without any severe phenotype, as shown in paper I. However, it has previously been shown that PARP1cd targeted to the mitochondria results in a severe phenotype with a dramatic shift in metabolic profile (VanLinden *et al*, 2015). Additionally, we observed that expression of the mitoPARP1cd construct had a severe effect on the fatty acid profile of the cells. None of these effects were observed in 293pexPARP1cd cells, indicating that if mitochondria are donating NAD they still retain or generate sufficient amounts to sustain their functions. In light of what one might call the "NAD-reservoir" hypothesis, one must consider also the possibility that the observed effects in 293mitoPARP1cd cells might be caused by ADP-ribosylation of proteins within mitochondria rather than increased NAD^+ consumption.

In one sense the observations made in this study open for more questions than they answer, and the interpretation is inevitably linked to the understanding of how the mitochondrial NAD pool is established and maintained. To determine the role of the mitochondrial NAD pool in counteracting alterations in NAD homeostasis, the mechanism behind the establishment and maintenance of this pool must be elucidated. Though so far not successful, the verification and identification of the responsible transporter would allow for investigations into its response in times of altered NAD homeostasis in the various pools. This could provide novel insight into the role of mitochondria in the regulation of cellular NAD-dependent processes.

Having observed that cells adapted to a chronic increase in compartmental NAD^+ consumption, we sought to investigate the effects of acute compartment specific NAD^+ depletion. To that end we temporarily halted the activity of PARP1cd, thereby restoring basal NAD^+ consumption rates is these cell. Upon resuming NAD^+ consumption, we observed hypoacetylation of H3K9 in cells with PARP1cd targeted to the mitochondria. Interestingly, this mirrored the findings observed upon inhibition of NAD biosynthesis by FK866. While it has been shown that acetylation state of □-tubulin K40 can be used as an inverse sensor for NAD^+ availability (Skoge *et al*, 2014), it is possible that the acetylation state of histones is reflecting a different effect. Inhibition of NamPRT by FK866 lowers SIRT1 activity, and since SIRT1 negatively regulates p53 dependent gene activation, (Luo *et al*, 2001; Kim *et al*, 2007; Vaziri *et al*, 2001), this leads to the transcription of p53 downstream target genes, such as p21 (Thakur *et al*, 2012) Expression of p21 promotes cell cycle arrest, a status that is strongly associated with hypoacetylation of H3 and H4 (Howe *et al*, 2001; Eckner, 2012; Koprinarova & Diederich, 2016; Karimian *et al*, 2016). Assuming this to be the causative effect behind lowered histone acetylation observed upon FK866 treatment, it could be inferred that chronic compartmentalized increase in NAD^+ consumption did not hinder cell cycle progression, while acute mitochondrial NAD^+ depletion did. Cell division is a highly energy-demanding process, fueled by ATP generated from metabolic NAD-dependent processes in mitochondria (Salazar-Roa & Malumbres, 2017). Illustrating the tight link between NAD availability and energy metabolism, it has been shown that cell death resulting from NAD^+ depletion is primarily caused by the cells inability to generate new ATP (Nagro *et al*, 2014). As alterations in energy availability trigger cell cycle checkpoints (Salazar-Roa & Malumbres, 2017), it is possible that lowered acetylation of H3K9 upon acute mitochondrial NAD^+ depletion reflects cell cycle arrest in response to energy depletion. This underlines the vital role of the mitochondrial NAD pool in cellular energy metabolism, and highlights the functionality of the compensatory response by the cell under chronic conditions.

4.3 The vital importance of regulating protein acetylation

Even though no change in protein acetylation was observed upon locally altered NAD homeostasis, the targeted expression of PARP1cd did results in significantly lowered cellular NAD^+ levels. Owing to the large number of competing NAD-dependent processes, there are mechanisms that tightly regulate distribution of NAD^+ amongst NAD^+ dependent enzymes (Houtkooper *et al*, 2010). Given the regulatory role of protein acetylation (Choudhary *et al*, 2014, 2009; Anderson *et al*, 2012), it was questioned whether the maintenance of protein acetylation occurred at the expense of other NAD-dependent processes. In line with this, altered sirtuin expression could reflect adjustments in the relative amount of NAD^+ allocated to deacetylation. Additionally, it has previously been shown that SIRT1 is capable of recruiting NMNAT1 to generate the NAD needed for deacetylation in a highly localized manner (Zhang *et al*, 2009). Thus, the produced NAD may be more readily available to sirtuins than other NAD-dependent enzymes.

Further supporting the notion that protein acetylation was maintained above other NAD-dependent processes, paper I found that increased NAD^+ consumption in mitochondria greatly affected the fatty acid profile of cells. This indicates that fatty acid oxidation was impaired, while paper II found the acetylation state of the mitochondrial protein MnSOD2 to remain unaltered under the same conditions. Increased ADP content in 293mitoPARP1cd cells observed in paper II could also suggest that increased NAD^+ consumption in mitochondria has an effect on energy metabolism. Thus, the cells appear to contain sufficient mitochondrial NAD^+ to maintain deacetylation and promote cell survival, but not to fully preserve all mitochondrial functions.

While NAD^+ availability and sirtuin activity are potent regulators of protein acetylation, the contribution of other KDACs and KATs to maintenance/alterations in protein acetylation should not be neglected. Additionally, KATs require acetyl-CoA. Like NAD, this is a compartmentalized metabolite, and there is functional interplay between NAD and acetyl-CoA signaling pathways which was not taken into account

in the present study (Peleg *et al*, 2016; Pietrocola *et al*, 2015; Drazic *et al*, 2016). Furthermore, in light of the changes in fatty acid profile resulting from increased NAD^+ consumption observed in paper I, it can be speculated that acetyl-groups on modified proteins may have been replaced by longer-chain acyl-groups. Such modifications are also targets for sirtuin activity (Feldman *et al*, 2013, 2015), but are not recognized by the antibodies used to assess acetylation state. To verify that the observed preservation of protein acetylation state is accurately reflecting sirtuin activity, it would therefore be relevant to determine the contribution of all non-sirtuin players in the regulation of protein acetylation, as well as the overall acylation state.

5. Concluding remarks and future perspectives

The findings presented in this dissertation shed new light on the role of the peroxisomal NAD pool, and the communication between subcellular NAD pools. In addition to extending the current understanding of inter-compartmental cooperation in the regulation of NAD-dependent processes, this work also raised several intriguing issues.

An important discovery from the presented work is the apparent cooperation between subcellular NAD pools with regards to NAD availability. While upregulated expression of SLC25A17 was postulated to facilitate the increased import of NAD^+ into peroxisomes, the remaining mechanisms involved are unclear. In order to understand how organelles communicate, adapt and cooperate, future studies should focus on identifying and characterizing the proteins involved in the influx and efflux of NAD across membranes, and the conditions that trigger this activity. For peroxisomes it would be relevant to investigate NUDT12, which in cleaving NAD(H) into NMN(H) and AMP could provide the cytosol, and adjacent organelles, with an important NAD precursor. For mitochondria, particular focus should be placed on the verification and identification of the elusive mitochondrial NAD transporter. As previously mentioned, this could provide a solid basis for investigations into how, when and why NAD is imported and exported from mitochondria. Additionally, deciphering the relative contribution of NAD import vs NMN transport and NMNAT3 activity to the total mitochondrial NAD content would better our understanding of how this organelle relates to the rest of the cell with regards to NAD availability

Importantly, this work suggests that cells adapt to increased NAD^+ consumption in the cytosol, peroxisomes and mitochondria by redistributing NAD, among the organelles. Still, the physical movement of NAD within the cell is yet to be verified. Additionally, while preliminary data shows that mitochondrial NAD^+ content is lowered in all cell lines, it remains unknown how much of the total NAD^+ depletion this accounts for, and whether other organelles are also depleted of NAD. By

continuously monitoring alterations in compartmental NAD content upon organellar PARP1cd expression (Cambronne *et al*, 2016) one could gain a better understanding of cells respond to NAD^+ depletion in each of the organelles. This, in turn, could provide valuable information into the relative importance of the subcellular NAD pools and the processes taking place within them. In addition to reallocation between subcellular NAD pools, it was postulated that NAD dependent processes were maintained in part owing to upregulated NAD biosynthesis. It would therefore be relevant to look into NAD turnover rate in the stably transfected cell lines compared to parental 293 cells.

Finally, one of the most striking outcomes in this study was the discovery that cells can maintain protein acetylation in times of lowered cellular NAD^+ content, and that a 30-40% decline of cellular NAD^+ can be compensated for by alterations in expression of sirtuins and NMNATs. Given that a hallmark of aging is a comparable decline in NAD^+ levels followed by hyperacetylation (Donmez & Guarente, 2010; Imai & Guarente, 2014; Guarente, 2000), the question becomes why similar mechanisms cannot compensate for age-related decline in NAD^+ levels. Part of the explanation probably lies in the difference between a compartment specific increased NAD^+ consumption compared to a global shift in both NAD^+ degradation and biosynthesis during aging (Schultz & Sinclair, 2016; Rajman *et al*, 2018). While aging is tied to lowered NAD content and decreased sirtuin activity, the relative contribution of the individual NAD pools to this effect remains unknown. By examining how NAD content of each individual NAD pool is affected by aging, and how the compartmentalized enzymes respond, one could get valuable insight into the order of cause and effect, and which pathways would serve best as therapeutic targets in the pursuit of anti-aging treatments.

6. References

Aarhus R, Graeff RM, Dickey DM, Walseth TF & Lee HC (1995) ADP-ribosyl cyclase and CD38 catalyze the synthesis of a calcium-mobilizing metabolite from NADP. *J. Biol. Chem.* **270:** 30327–30333

AbdelRaheim SR, Cartwright JL, Gasmi L & McLennan AG (2001) The NADH diphosphatase encoded by the Saccharomyces cerevisiae NPY1 nudix hydrolase gene is located in peroxisomes. *Arch. Biochem. Biophys.* **388:** 18-24

Abdelraheim SR, Spiller DG & McLennan AG (2017) Mouse Nudt13 is a Mitochondrial Nudix Hydrolase with NAD(P)H Pyrophosphohydrolase Activity. *Protein J.* **36:** 425-432

AbdelRaheim SR, Spiller DG & McLennan AG (2003) Mammalian NADH diphosphatases of the Nudix family: cloning and characterization of the human peroxisomal NUDT12 protein. *Biochem. J.* **374:** 329-335

Abe Y, Honsho M, Nakanishi H, Taguchi R & Fujiki Y (2014) Very-long-chain polyunsaturated fatty acids accumulate in phosphatidylcholine of fibroblasts from patients with Zellweger syndrome and acyl-CoA oxidase1 deficiency. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids.* **1841:** 610-619

Agrimi G, Russo A, Scarcia P & Palmieri F (2012) The human gene *SLC25A17* encodes a peroxisomal transporter of coenzyme A, FAD and NAD $^{+}$. *Biochem. J.* **443:** 241–247

Al-Saryi NA, Al-Hejjaj MY, van Roermund CWT, Hulmes GE, Ekal L, Payton C, Wanders RJA & Hettema EH (2017) Two NAD-linked redox shuttles maintain the peroxisomal redox balance in Saccharomyces cerevisiae. *Sci. Rep.* **7:** 11868

Alano CC, Tran A, Tao R, Ying W, Karliner JS & Swanson RA (2007) Differences among cell types in NAD+ compartmentalization: A comparison of neurons, astrocytes, and cardiac myocytes. In *Journal of Neuroscience Research.* **85:** 3378-3385

Allfrey V, Faulkner R & Mirsky A (1964) Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. *Proc. Natl. Acad. Sci. U. S. A.* **51:** 786–794

Anderson K a, Hirschey MD & Matthew D (2012) Mitochondrial protein acetylation regulates metabolism. *Essays Biochem.* **52:** 23–35

Anderson RM, Bitterman KJ, Wood JG, Medvedik O & Sinclair DA (2003) Nicatinamide and PNC1 govern lifespan extension by calorie restriction in Saccharomyces cerevisiae. *Nature* **423:** 181–185

Antonenkov VD & Hiltunen JK (2012) Transfer of metabolites across the peroxisomal membrane. *Biochim. Biophys. Acta - Mol. Basis Dis.***1822:** 1374-1386

Arif M, Kumar GVP, Narayana C & Kundu TK (2007) Autoacetylation induced specific structural changes in histone acetyltransferase domain of p300: Probed by surface enhanced Raman spectroscopy. *J. Phys. Chem. B.* **111:** 11877-11879

Aykroyd WR (1914) The Etiology of Pellagra. *Public health rep.* **29**

Bai P, Cantó C, Oudart H, Brunyánszki A, Cen Y, Thomas C, Yamamoto H, Huber A, Kiss B, Houtkooper RH, Schoonjans K, Schreiber V, Sauve AA, Menissier-De Murcia J & Auwerx J (2011) PARP-1 inhibition increases mitochondrial metabolism through SIRT1 activation. *Cell Metab.* **13:** 461–468

Barbosa MTP, Soares SM, Novak CM, Sinclair D, Levine JA, Aksoy P & Chini EN (2007) The enzyme CD38 (a NAD glycohydrolase, EC 3.2.2.5) is necessary for the development of diet-induced obesity. *FASEB J.* **21**: 3629-3639

Barkauskaite E, Brassington A, Tan ES, Warwicker J, Dunstan MS, Banos B, Lafite P, Ahel M, Mitchison TJ, Ahel I & Leys D (2013) Visualization of poly(ADP-ribose) bound to PARG reveals inherent balance between exo- and endo-glycohydrolase activities. *Nat. Commun.* **4:** 2164

Baumgart E, Fahimi HD, Stich A & Völkl A (1996) L-lactate dehydrogenase A4- and A3B isoforms are bona fide peroxisomal enzymes in rat liver: Evidence for involvement in intraperoxisomal nadh reoxidation. *J. Biol. Chem.* **271:** 3846-3855

Berger F, Lau C, Dahlmann M & Ziegler M (2005) Subcellular compartmentation and differential catalytic properties of the three human nicotinamide mononucleotide adenylyltransferase isoforms. *J. Biol. Chem.* **280:** 36334–36341

Berndsen CE & Denu JM (2008) Catalysis and substrate selection by histone/protein lysine acetyltransferases. *Curr. Opin. Struct. Biol.* **18:** 682–689

Berthelier V, Tixier JM, Muller-Steffner H, Schuber F & Deterre P (1998) Human CD38 is an authentic NAD(P)+ glycohydrolase. *Biochem. J.* **330:** 1383–1390

Bharathi SS, Zhang Y, Mohsen AW, Uppala R, Balasubramani M, Schreiber E, Uechi G, Beck ME, Vockley J, Rardin MJ, Verdin E, Gibson BW, Hirschey MD & Goetzman ES (2013) Sirtuin 3 (SIRT3) protein regulates long-chain acyl-CoA dehydrogenase by deacetylating conserved lysines near the active site. *J. Biol. Chem.* **288:** 33837-33847

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

Bilan V, Selevsek N, Kistemaker HA V., Abplanalp J, Feurer R, Filippov D V. & Hottiger MO (2017) New Quantitative Mass Spectrometry Approaches Reveal Different ADP-ribosylation Phases Dependent On the Levels of Oxidative Stress. *Mol. Cell. Proteomics.* **16:** 949-958

Billington RA, Genazzani AA, Travelli C & Condorelli F (2008) NAD depletion by FK866 induces autophagy. *Autophagy.* **4**: 385:387

Bitterman KJ, Anderson RM, Cohen HY, Latorre-Esteves M & Sinclair D a. (2002) Inhibition of silencing and accelerated aging by nicotinamide, a putative negative regulator of yeast Sir2 and human SIRT1. *J. Biol. Chem.* **277:** 45099–45107

Bonicalzi M-E, Haince J-F, Droit A & Poirier GG (2005) Poly-ADP-ribosylation in health and disease. *Cell. Mol. Life Sci. C.* **62:** 739–750

Burgos ES & Schramm VL (2008) Weak coupling of ATP hydrolysis to the chemical equilibrium of human nicotinamide phosphoribosyltransferase. *Biochemistry* **47:** 11086–11096

Camacho-Pereira J, Tarragó MG, Chini CCS, Nin V, Escande C, Warner GM, Puranik AS, Schoon RA, Reid JM, Galina A & Chini EN (2016) CD38 Dictates Age-Related NAD Decline and Mitochondrial Dysfunction through an SIRT3-Dependent Mechanism. *Cell Metab.* **23:** 1127–1139

Cambronne XA, Stewart ML, Kim D, Jones-Brunette AM, Morgan RK, Farrens DL, Cohen MS & Goodman RH (2016) Biosensor reveals multiple sources for mitochondrial NAD+. *Science.* **352:** 1474–1477

Cantó C, Gerhart-Hines Z, Feige JN, Lagouge M, Noriega L, Milne JC, Elliott PJ, Puigserver P & Auwerx J (2009) AMPK regulates energy expenditure by modulating NAD + metabolism and SIRT1 activity. *Nature.* **458:** 1056-1060

Cantó C, Houtkooper RH, Pirinen E, Youn DY, Oosterveer MH, Cen Y, Fernandez-Marcos PJ, Yamamoto H, Andreux PA, Cettour-Rose P, Gademann K, Rinsch C, Schoonjans K, Sauve AA & Auwerx J (2012) The NAD+ precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet-induced obesity. *Cell Metab.* **15:** 838–847

Caron C, Boyault C & Khochbin S (2005) Regulatory cross-talk between lysine acetylation and ubiquitination: role in the control of protein stability. *BioEssays* **27:** 408–415

Carreras-Puigvert J, Zitnik M, Jemth AS, Carter M, Unterlass JE, Hallström B, Loseva O, Karem Z, Calderón-Montanõ JM, Lindskog C, Edqvist PH, Matuszewski DJ, Ait Blal H, Berntsson RPA, Häggblad M, Martens U, Studham M, Lundgren B, Wählby C, Sonnhammer ELL, Lundberg E, Stenmark P & Helleday T (2017) A comprehensive structural, biochemical and biological profiling of the human NUDIX hydrolase family. *Nat. Commun.* **8:** 1541

Cea M, Cagnetta A, Patrone F, Nencioni A, Gobbi M & Anderson KC (2013) Intracellular NAD^+ depletion induces autophagic death in multiple myeloma cells. *Autophagy.* **9:** 410-412

De Cecco M, Criscione SW, Peterson AL, Neretti N, Sedivy JM & Kreiling JA (2013) Transposable elements become active and mobile in the genomes of aging mammalian somatic tissues. *Aging.* **5:** 867–883

Chambon P, Weill JD & Mandel P (1963) Nicotinamide mononucleotide activation of a new DNA-dependent polyadenylic acid synthesizing nuclear enzyme. *Biochem. Biophys. Res. Commun.* **11:** 39–43

Chen D, Steele AD, Lindquist S & Guarente L (2005) Medicine: Increase in activity during calorie restriction requires Sirt1. *Science* . **310:** 1641

Choudhary C, Kumar C, Gnad F, Nielsen ML, Rehman M, Walther TC, Olsen J V. & Mann M (2009) Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science.*. **325:** 834–840

Choudhary C, Weinert BT, Nishida Y, Verdin E & Mann M (2014) The growing landscape of lysine acetylation links metabolism and cell signalling. *Nat. Rev. Mol. Cell Biol.* **15:** 536–550

Cohen HY, Miller C, Bitterman KJ, Wall NR, Hekking B, Kessler B, Howitz KT, Gorospe M, De Cabo R & Sinclair DA (2004) Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. *Science.* **305:** 390–392

Dali-Youcef N, Lagouge M, Froelich S, Koehl C, Schoonjans K & Auwerx J (2007) Sirtuins: The 'magnificent seven', function, metabolism and longevity. *Ann. Med.* **39:** 335–345

Daniels CM, Thirawatananond P, Ong SE, Gabelli SB & Leung AKL (2015) Nudix hydrolases degrade protein-conjugated ADP-ribose. *Sci. Rep.* **5:** 18271

Davila A, Liu L, Chellappa K, Redpath P, Nakamaru-Ogiso E, Paolella LM, Zhang Z, Migaud ME, Rabinowitz JD & Baur JA (2018) Nicotinamide adenine dinucleotide is transported into mammalian mitochondria. *Elife.* **7:** e33246

Dellinger RW, Santos SR, Morris M, Evans M, Alminana D, Guarente L & Marcotulli E (2017) Repeat dose NRPT (nicotinamide riboside and pterostilbene) increases NAD+ levels in humans safely and sustainably: a randomized, double-blind, placebo-controlled study. *npj Aging Mech. Dis.* **3:** 17

Del Río LA & López-Huertas E (2016) ROS generation in peroxisomes and its role in cell signaling. *Plant Cell Physiol.* **57:** 1364-1376

de Murcia JM, Niedergang C, Trucco C, Ricoul M, Dutrillaux B, Mark M, Oliver FJ, Masson M, Dierich A, LeMeur M, Walztinger C, Chambon P & de Murcia G (1997) Requirement of poly(ADP-ribose) polymerase in recovery from DNA damage in mice and in cells. *Proc. Natl. Acad. Sci. U. S. A.* **94:** 7303-7307

Di Lisa F (2002) Mitochondrial Contribution in the Progression of Cardiac Ischemic Injury. *IUBMB Life* **52:** 255–261

Di Lisa F, Menabò R, Canton M, Barile M & Bernardi P (2001) Opening of the Mitochondrial Permeability Transition Pore Causes Depletion of Mitochondrial and Cytosolic NAD+ and Is a Causative Event in the Death of Myocytes in Postischemic Reperfusion of the Heart. *J. Biol. Chem.* **276:** 2571-2575

Dölle C, Niere M, Lohndal E & Ziegler M (2010) Visualization of subcellular NAD pools and intra-organellar protein localization by poly-ADP-ribose formation. *Cell. Mol. Life Sci.* **67:** 433–443

Donmez G & Guarente L (2010) Aging and disease: Connections to sirtuins. *Aging Cell* **9:** 285–290

Drazic A, Myklebust LM, Ree R & Arnesen T (2016) The world of protein acetylation. *Biochim. Biophys. Acta - Proteins Proteomics.* **1864:** 1372-1401

Eckner R (2012) p53-dependent growth arrest and induction of p21: A critical role for PCAF-mediated histone acetylation. *Cell Cycle* **11:** 2591-2592

Escande C, Nin V, Price NL, Capellini V, Gomes AP, Barbosa MT, O'Neil L, White TA, Sinclair DA, Chini EN (2013) Flavonoid apigenin is an inhibitor of the NAD+ase CD38: Implications for cellular NAD+ metabolism, protein acetylation, and treatment of metabolic syndrome. *Diabetes* **62:** 1084–1093

Feldman JL, Baeza J & Denu JM (2013) Activation of the protein deacetylase SIRT6 by long-chain fatty acids and widespread deacylation by Mammalian Sirtuins. *J. Biol. Chem.* **288:** 31350-31356

Feldman JL, Dittenhafer-Reed KE & Denu JM (2012) Sirtuin catalysis and regulation. *J. Biol. Chem.* **287:** 42419–42427

Feldman JL, Dittenhafer-Reed KE, Kudo N, Thelen JN, Ito A, Yoshida M & Denu JM (2015) Kinetic and structural basis for Acyl-group selectivity and NAD^+ dependence in sirtuin-catalyzed deacylation. *Biochemistry.* **54:** 3037-3050

Felici R, Lapucci A, Cavone L, Pratesi S, Berlinguer-Palmini R & Chiarugi A (2015) Pharmacological NAD-Boosting Strategies Improve Mitochondrial Homeostasis in Human Complex I-Mutant Fibroblasts. *Mol. Pharmacol.* **87:** 965–971

Ferdinandusse S, Denis S, Mooyer PAW, Dekker C, Duran M, Soorani-Lunsing RJ, Boltshauser E, Macaya A, Gärtner J, Majoie CBLM, Barth PG, Wanders RJA & Poll-The BT (2006) Clinical and biochemical spectrum of D-bifunctional protein deficiency. *Ann. Neurol.* **59:** 92-104

Finkel T, Deng CX & Mostoslavsky R (2009) Recent progress in the biology and physiology of sirtuins. *Nature* **460:** 587–591

Fontana L, Partridge L & Longo VD (2010) Extending healthy life span-from yeast to humans. *Science.* **328:** 321–326

Fouquerel E & Sobol RW (2014) ARTD1 (PARP1) activation and NAD+ in DNA repair and cell death. *DNA Repair (Amst).* **23:** 27-32

Fransen M, Lismont C & Walton P (2017) The peroxisome-mitochondria connection: How and why? *Int. J. Mol. Sci.* **18:** 1126

Frye RA (1999) Characterization of five human cDNAs with homology to the yeast SIR2 gene: Sir2-like proteins (Sirtuins) metabolize NAD and may have protein ADP-ribosyltransferase activity. *Biochem. Biophys. Res. Commun.* **260:** 273–279

Gansen A, Tóth K, Schwarz N & Langowski J (2015) Opposing roles of H3- and H4-acetylation in the regulation of nucleosome structure - A FRET study. *Nucleic Acids Res.* **43:** 1433–1443

Gasmi L & McLennan AG (2001) The mouse Nudt7 gene encodes a peroxisomal nudix hydrolase specific for coenzyme A and its derivatives. *Biochem. J.* **357:** 33-38

Gasser A, Glassmeier G, Fliegert R, Langhorst MF, Meinke S, Hein D, Krüger S, Weber K, Heiner I, Oppenheimer N, Schwarz JR & Guse AH (2006) Activation of T cell calcium influx by the second messenger ADP-ribose. *J. Biol. Chem.* **281:** 2489–2496

Geisbrecht B V. & Gould SJ (1999) The human PICD gene encodes a cytoplasmic and peroxisomal NADP+- dependent isocitrate dehydrogenase. *J. Biol. Chem.* **274:** 30527-30533

Glozak M a., Sengupta N, Zhang X & Seto E (2005) Acetylation and deacetylation of non-histone proteins. *Gene* **363:** 15–23

Goldberger J, Waring CH & Willets DG (1915) A test of diet in the prevention of pellagra. *Public health rep.* **30:** 3117-3131

Gomes AP, Price NL, Ling AJY, Moslehi JJ, Montgomery MK, Rajman L, White JP, Teodoro JS, Wrann CD, Hubbard BP, Mercken EM, Palmeira CM, De Cabo R, Rolo AP, Turner N, Bell EL & Sinclair DA (2013) Declining NAD+ induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during aging. *Cell* **155:** 1624–1638

Graeff R, Liu Q, Kriksunov IA, Kotaka M, Oppenheimer N, Hao Q & Lee HC (2009) Mechanism of cyclizing NAD to cyclic ADP-ribose by ADP-ribosyl cyclase and CD38. *J. Biol. Chem.* **284:** 27629-27636

Grube K & Burkle A (1992) Poly(ADP-ribose) polymerase activity in mononuclear leukocytes of 13 mammalian species correlates with species-specific life span. *Proc. Natl. Acad. Sci.* **89:** 11759–11763

Guan KL & Xiong Y (2011) Regulation of intermediary metabolism by protein acetylation. *Trends Biochem. Sci.* **36:** 108-116

Guan Y, Wang S-R, Huang X-Z, Xie Q, Xu Y-Y, Shang D & Hao C-M (2017) Nicotinamide Mononucleotide, an NAD $^{+}$ Precursor, Rescues Age-Associated Susceptibility to AKI in a Sirtuin 1–Dependent Manner. *J. Am. Soc. Nephrol.* **28:** 2337-2352

Guarente L (2000) Sir2 links chromatin silencing, metabolism, and aging. *Genes Dev.* **14:** 1021–1026

Gulshan M, Yaku K, Okabe K, Mahmood A, Sasaki T, Yamamoto M, Hikosaka K, Usui I, Kitamura T, Tobe K & Nakagawa T (2018) Overexpression of Nmnat3 efficiently increases NAD and NGD levels and ameliorates age-associated insulin resistance. *Aging Cell.* **17:** e12798

Guse AH (2015) Calcium mobilizing second messengers derived from NAD. *Biochim. Biophys. Acta* **1854:** 1132–1137

Haberland M, Montgomery RL & Olson EN (2009) The many roles of histone deacetylases in development and physiology: Implications for disease and therapy. *Nat. Rev. Genet.* **10:** 32–42

Hallows WC, Lee S & Denu JM (2006) Sirtuins deacetylate and activate mammalian acetyl-CoA synthetases. *Proc. Natl. Acad. Sci. U. S. A.* **103:** 10230–10235

Hara N, Yamada K, Shibata T, Osago H, Hashimoto T & Tsuchiya M (2007) Elevation of Cellular NAD Levels by Nicotinic Acid and Involvement of Nicotinic Acid Phosphoribosyltransferase in Human Cells. *J. Biol. Chem.* **282:** 24574–24582

Hottiger MO, Hassa PO, Lüscher B, Schüler H & Koch-Nolte F (2010) Toward a unified nomenclature for mammalian ADP-ribosyltransferases. *Trends Biochem. Sci.* **35:** 208–219

Houtkooper RH, Cantó C, Wanders RJ & Auwerx J (2010) The secret life of NAD+: An old metabolite controlling new metabolic signaling pathways. *Endocr. Rev.* **31:** 194–223

Howard M, Grimaldi JC, Bazan JF, Lund FE, Santos-Argumedo L, Parkhouse RM, Walseth TF & Lee HC (1993) Formation and hydrolysis of cyclic ADP-ribose catalyzed by lymphocyte antigen CD38. *Science* **262:** 1056–1059

Howe L, Auston D, Grant P, John S, Cook RG, Workman JL & Pillus L (2001) Histone H3 specific acetyltransferases are essential for cell cycle progression. *Genes Dev.* **15:** 3144-3154

Imai S ichiro & Guarente L (2014) NAD+ and sirtuins in aging and disease. *Trends Cell Biol.* **24:** 464–471

Imai SI, Armstrong CM, Kaeberlein M & Guarente L (2000) Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* **403:** 795–800

Janke C & Montagnac G (2017) Causes and Consequences of Microtubule Acetylation. *Curr. Biol.* **27:** 1287-1292

Jankevicius G, Hassler M, Golia B, Rybin V, Zacharias M, Timinszky G & Ladurner AG (2013) A family of macrodomain proteins reverses cellular mono-ADP-ribosylation. *Nat. Struct. Mol. Biol.* **20:** 508-514

Jing E, Gesta S & Kahn CR (2007) SIRT2 Regulates Adipocyte Differentiation through FoxO1 Acetylation/Deacetylation. *Cell Metab.* **6:** 105-114

Kane DA (2014) Lactate oxidation at the mitochondria: A lactate-malate-aspartate shuttle at work. *Front. Neurosci.* **8:** 366

Karimian A, Ahmadi Y & Yousefi B (2016) Multiple functions of p21 in cell cycle, apoptosis and transcriptional regulation after DNA damage. *DNA Repair (Amst).* **42:** 63-71

Kemp S, Berger J & Aubourg P (2012) X-linked adrenoleukodystrophy: Clinical, metabolic, genetic and pathophysiological aspects. *Biochim. Biophys. Acta - Mol. Basis Dis.* **1822:** 1465-1474

Kim EJ, Kho JH, Kang MR & Um SJ (2007) Active Regulator of SIRT1 Cooperates with SIRT1 and Facilitates Suppression of p53 Activity. *Mol. Cell.* **28:** 277-290

Kim G-W, Gocevski G, Wu C-J YX-J (2010) Dietary, Metabolic, and Potentially Environmental Modulation of the Lysine Acetylation Machinery. *Int. J. Cell Biol.*

Kim SC, Sprung R, Chen Y, Xu Y, Ball H, Pei J, Cheng T, Kho Y, Xiao H, Xiao L, Grishin N V., White M, Yang XJ & Zhao Y (2006) Substrate and Functional Diversity of Lysine Acetylation Revealed by a Proteomics Survey. *Mol. Cell* **23:** 607–618

Kirchberger T & Guse AH (2013) Measuring CD38 (ADP-ribosyl Cyclase/Cyclic ADP-ribose hydrolase) activity by reverse-phase HPLC. *Cold Spring Harb. Protoc.* **8:** 569–573

Kirkman HN & Gaetani GF (1984) Catalase: a tetrameric enzyme with four tightly bound molecules of NADPH. *Proc. Natl. Acad. Sci. U. S. A.* **81:** 4343-4347

Kirkman HN, Rolfo M, Ferraris AM & Gaetani GF (1999) Mechanisms of protection of catalase by NADPH: Kinetics and stoichiometry. *J. Biol. Chem.* **274:** 13908-13914

Koprinarova M & Diederich MS and M (2016) Role of Histone Acetylation in Cell Cycle Regulation. *Curr. Top. Med. Chem.* **16:** 732–744

Landry J, Sutton a, Tafrov ST, Heller RC, Stebbins J, Pillus L & Sternglanz R (2000) The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. *Proc. Natl. Acad. Sci. U. S. A.* **97:** 5807–5811

Langelier MF, Planck JL, Roy S & Pascal JM (2012) Structural basis for DNA damage-dependent poly(ADP-ribosyl)ation by human PARP-1. *Science.* **336:** 728–732

Langelier MF, Servent KM, Rogers EE & Pascal JM (2008) A third zinc-binding domain of human poly(ADP-ribose) polymerase-1 coordinates DNA-dependent enzyme activation. *J. Biol. Chem.* **283:** 4105–4114

Lau C, Dölle C, Gossmann TI, Agledal L, Niere M & Ziegler M (2010) Isoform-specific targeting and interaction domains in human nicotinamide mononucleotide adenylyltransferases. *J. Biol. Chem.* **285:** 18868–18876

Lee HC (2012) Cyclic ADP-ribose and nicotinic acid adenine dinucleotide phosphate (NAADP) as messengers for calcium mobilization. *J. Biol. Chem.* **287:** 31633–31640

Li W, Prazak L, Chatterjee N, Grüninger S, Krug L, Theodorou D & Dubnau J (2013) Activation of transposable elements during aging and neuronal decline in Drosophila. *Nat. Neurosci.* **16:** 529–531

Lin SJ, Defossez PA & Guarente L (2000) Requirement of NAD and SIR2 for life-span extension by calorie restriction in saccharomyces cerevisiae. *Science.* **289:** 2126–2128

Lin Y yi, Lu J ying, Zhang J, Walter W, Dang W, Wan J, Tao SC, Qian J, Zhao Y, Boeke JD, Berger SL & Zhu H (2009) Protein Acetylation Microarray Reveals that NuA4 Controls Key Metabolic Target Regulating Gluconeogenesis. *Cell.* **136:** 1073-1084

Lismont C, Nordgren M, Van Veldhoven PP & Fransen M (2015) Redox interplay between mitochondria and peroxisomes. *Front. Cell Dev. Biol.* **3:** 35

Luo J, Nikolaev AY, Imai S, Chen D, Su F, Shiloh A, Guarente L & Gu W (2001) Negative control of p53 by Sir2alpha promotes cell survival under stress. *Cell.* 107: 137-148

Marmorstein R & Roth SY (2001) Histone acetyltransferases: Function, structure, and catalysis. *Curr. Opin. Genet. Dev.* **11:** 155–161

McCay CM, Crowell MF & Maynard LA (1935) The effect of retarded growth upon the length of life span and upon the ultimate body size. *J. Nutr.* **5:** 63–79.

Ménissier de Murcia J, Ricoul M, Tartier L, Niedergang C, Huber A, Dantzer F, Schreiber V, Amé JC, Dierich A, LeMeur M, Sabatier L, Chambon P & De Murcia G (2003) Functional interaction between PARP-1 and PARP-2 in chromosome stability and embryonic development in mouse. *EMBO J.* **22:** 2255-2263

Meyer-Ficca ML, Meyer RG, Coyle DL, Jacobson EL & Jacobson MK (2004) Human poly(ADP-ribose) glycohydrolase is expressed in alternative splice variants yielding isoforms that localize to different cell compartments. *Exp. Cell Res.* **297:** 521-532

Meyer RG, Meyer-Ficca ML, Whatcott CJ, Jacobson EL & Jacobson MK (2007) Two small enzyme isoforms mediate mammalian mitochondrial poly(ADP-ribose) glycohydrolase (PARG) activity. *Exp. Cell Res.* **313:** 2920-2936

Mitchell SJ, Bernier M, Aon MA, Cortassa S, Kim EY, Fang EF, Palacios HH, Ali A, Navas-Enamorado I, Di Francesco A, Kaiser TA, Waltz TB, Zhang N, Ellis JL, Elliott PJ, Frederick DW, Bohr VA, Schmidt MS, Brenner C, Sinclair DA, Sauve AA, Baur JA, de Cabo, R (2018) Nicotinamide Improves Aspects of Healthspan, but Not Lifespan, in Mice. *Cell Metab.* **27:** 667-676

Morales V & Richard-Foy H (2000) Role of Histone N-Terminal Tails and Their Acetylation in Nucleosome Dynamics. *Mol. Cell. Biol.* **20:** 7230–7237

Mouchiroud L, Houtkooper RH & Auwerx J (2013) NAD+ metabolism: A therapeutic target for age-related metabolic disease. *Crit. Rev. Biochem. Mol. Biol.* **48:** 397–408

Nagro C Del, Xiao Y, Rangell L, Reichelt M & O'Brien T (2014) Depletion of the Central Metabolite NAD leads to oncosismediated cell death. *J. Biol. Chem.* **289:** 35182–35192

Niere M, Kernstock S, Koch-Nolte F & Ziegler M (2008) Functional localization of two poly(ADP-ribose)-degrading enzymes to the mitochondrial matrix. *Mol. Cell. Biol.* **28:** 814–824

Niere M, Mashimo M, Agledal L, Dölle C, Kasamatsu A, Kato J, Moss J & Ziegler M (2012) ADP-ribosylhydrolase 3 (ARH3), not poly(ADP-ribose) glycohydrolase (PARG) isoforms, is responsible for degradation of mitochondrial matrix-associated poly(ADP-ribose). *J. Biol. Chem.* **287:** 16088-16102

Nikiforov A, Dölle C, Niere M & Ziegler M (2011) Pathways and subcellular compartmentation of NAD biosynthesis in human cells: From entry of extracellular precursors to mitochondrial NAD generation. *J. Biol. Chem.* **286:** 21767–21778

Nikiforov A, Kulikova V & Ziegler M (2015) The human NAD metabolome: Functions, metabolism and compartmentalization. *Crit. Rev. Biochem. Mol. Biol.* **50:** 284–297

Nishino T, Okamoto K, Eger BT, Pai EF & Nishino T (2008) Mammalian xanthine oxidoreductase - Mechanism of transition from xanthine dehydrogenase to xanthine oxidase. *FEBS J.* **275:** 3278-3289

Nishizuka Y & Hayaishi O (1963) Studies on the Biosynthesis of Nicotinamide Adenine Dinucleotide: I. Enzymatic synthesis of niacin ribonucleotides from 3-hydroxyanthranilic acid in mammalian tissues.. *J. Biol. Chem.* **238:** 3369–3377

Nishizuka Y & Hayaishi O (1963) Enzymic synthesis of niacin nucleotides from 3-hydroxyanthranilic acid. *J. Biol. Chem.* **238:** 483–485

North BJ, Marshall BL, Borra MT, Denu JM & Verdin E (2003) The human Sir2 ortholog, SIRT2, is an NAD+-dependent tubulin deacetylase. *Mol. Cell* **11:** 437–444

Norton VG, Imai BS, Yau P & Bradbury EM (1989) Histone acetylation reduces nucleosome core particle linking number change. *Cell* **57:** 449–457

Norton VG, Marvin KW, Yau P & Bradbury EM (1990) Nucleosome linking number change controlled by acetylation of histones H3 and H4. *J. Biol. Chem.* **265:** 19848–19852

Ofman R, Speijer D, Leen R & Wanders RJA (2006) Proteomic analysis of mouse kidney peroxisomes: identification of RP2p as a peroxisomal nudix hydrolase with acyl-CoA diphosphatase activity. *Biochem. J.* 393: 537-543

Ozden O, Park SH, Kim HS, Jiang H, Coleman MC, Spitz DR & Gius D (2011) Acetylation of MnSOD directs enzymatic activity responding to cellular nutrient status or oxidative stress. *Aging.* **3:** 102–107

Palazzo L, Thomas B, Jemth A-S, Colby T, Leidecker O, Feijs KLH, Zaja R, Loseva O, Puigvert JC, Matic I, Helleday T & Ahel I (2015) Processing of protein ADP-ribosylation by Nudix hydrolases. *Biochem. J.* **468:** 293-301

Palmieri F, Rieder B, Ventrella A, Blanco E, Do PT, Nunes-Nesi A, Trauth a U, Fiermonte G, Tjaden J, Agrimi G, Kirchberger S, Paradies E, Fernie AR & Neuhaus HE (2009) Molecular identification and functional characterization of Arabidopsis thaliana mitochondrial and chloroplastic NAD+ carrier proteins. *J. Biol. Chem.* 284: 31249-31259

Peleg S, Feller C, Ladurner AG & Imhof A (2016) The Metabolic Impact on Histone Acetylation and Transcription in Ageing. *Trends Biochem. Sci.* **41:** 700–711

Peleg S, Sananbenesi F, Zovoilis A, Burkhardt S, Bahari-Javan S, Agis-Balboa RC, Cota P, Wittnam JL, Gogol-Doering A, Opitz L, Salinas-Riester G, Dettenhofer M, Kang H, Farinelli L, Chen W & Fischer A (2010) Altered histone acetylation is associated with age-dependent memory impairment in mice. *Science.* **328:** 753–756

Pietrocola F, Galluzzi L, Bravo-San Pedro JM, Madeo F & Kroemer G (2015) Acetyl coenzyme A: A central metabolite and second messenger. *Cell Metab.* **21:** 805–821

Poirier Y, Antonenkov VD, Glumoff T & Hiltunen JK (2006) Peroxisomal β-oxidation-A metabolic pathway with multiple functions. *Biochim. Biophys. Acta - Mol. Cell Res.* **1763:** 1413-1426

Preiss J & Handler P (1958a) Biosynthesis of diphosphopyridine nucleotide. I. Identification of intermediates. *J. Biol. Chem.* **233:** 488–492

Preiss J & Handler P (1958b) Biosynthesis of diphosphopyridine nucleotide. II. Enzymatic aspects. *J. Biol. Chem.* **233:** 493–500

Rack JGM, Perina D & Ahel I (2016) Macrodomains: Structure, Function, Evolution, and Catalytic Activities. *Annu. Rev. Biochem.* **85:** 431-454

Rajman L, Chwalek K & Sinclair DA (2018) Therapeutic Potential of NAD-Boosting Molecules: The in vivo Evidence. *Cell Metab.* **27:** 529–547

Ritter H, Koch-Nolte F, Marquez VE & Schulz GE (2003) Substrate binding and catalysis of ecto-ADP-ribosyltransferase 2.2 from rat. *Biochemistry* **42:** 10155–10162

Rosenthal F, Feijs KLH, Frugier E, Bonalli M, Forst AH, Imhof R, Winkler HC, Fischer D, Caflisch A, Hassa PO, Lüscher B & Hottiger MO (2013) Macrodomain-containing proteins are new mono-ADP-ribosylhydrolases. *Nat. Struct. Mol. Biol.* **20:** 502-507

Rottensteiner H & Theodoulou FL (2006) The ins and outs of peroxisomes: Co-ordination of membrane transport and peroxisomal metabolism. *Biochim. Biophys. Acta - Mol. Cell Res.* **1763:** 1527-1540

Rowen JW & Kornberg A (1951) The phosphorolysis of nicotinamide riboside. *J. Biol. Chem.* **193:** 497–507

Ruf A, Rolli V, De Murcia G & Schulz GE (1998) The mechanism of the elongation and branching reaction of Poly(ADP-ribose) polymerase as derived from crystal structures and mutagenesis. *J. Mol. Biol.* **278:** 57–65

Ryu KW, Nandu T, Kim J, Challa S, DeBerardinis RJ & Kraus WL (2018) Metabolic regulation of transcription through compartmentalized NAD+ biosynthesis. *Science.* **360**

Sadoul K, Wang J, Diagouraga B & Khochbin S (2011) The tale of protein lysine acetylation in the cytoplasm. *J. Biomed. Biotechnol.* **2011**

Saksela M & Raivio KO (1996) Cloning and expression in vitro of human xanthine dehydrogenase/oxidase. *Biochem. J.* **315:** 235–239

Salazar-Roa M & Malumbres M (2017) Fueling the Cell Division Cycle. *Trends Cell Biol.* **27:** 69-81

Schrader M & Fahimi HD (2006) Peroxisomes and oxidative stress. *Biochim. Biophys. Acta - Mol. Cell Res.* **1763:** 1755-1766

Schreiber V, Dantzer F, Amé JC & De Murcia G (2006) Poly(ADP-ribose): Novel functions for an old molecule. *Nat. Rev. Mol. Cell Biol.* **7:** 517-528

Schultz MB & Sinclair DA (2016) Why NAD+ Declines during Aging: It's Destroyed. *Cell Metab.* **23:** 965–966

Schwer B, Bunkenborg J, Verdin RO, Andersen JS & Verdin E (2006) Reversible lysine acetylation controls the activity of the mitochondrial enzyme acetyl-CoA synthetase 2. *Proc. Natl. Acad. Sci.* **103:** 10224–10229

Sharifi R, Morra R, Denise Appel C, Tallis M, Chioza B, Jankevicius G, Simpson MA, Matic I, Ozkan E, Golia B, Schellenberg MJ, Weston R, Williams JG, Rossi MN, Galehdari H, Krahn J, Wan A, Trembath RC, Crosby AH, Ahel D, Hay R, Ladurner AG, Timinszky G, Williams RS, Ahel I (2013) Deficiency of terminal ADP-ribose protein glycohydrolase TARG1/C6orf130 in neurodegenerative disease. *EMBO J.* **32:** 1225-1237

Skoge RH, Dölle C & Ziegler M (2014) Regulation of SIRT2-dependent α-tubulin deacetylation by cellular NAD levels. *DNA Repair (Amst).* **23:** 33–38

Slade D, Dunstan MS, Barkauskaite E, Weston R, Lafite P, Dixon N, Ahel M, Leys D & Ahel I (2011) The structure and catalytic mechanism of a poly(ADP-ribose) glycohydrolase. *Nature.* **477:** 616-620

Song J, Ke SF, Zhou CC, Zhang SL, Guan YF, Xu TY, Sheng CQ, Wang P & Miao CY (2014) Nicotinamide phosphoribosyltransferase is required for the calorie restriction-mediated improvements in oxidative stress, mitochondrial biogenesis, and metabolic adaptation. *Journals Gerontol. - Ser. A Biol. Sci. Med. Sci.* **69:** 44–57

Sun N, Youle RJ & Finkel T (2016) The Mitochondrial Basis of Aging. *Mol. Cell* **61:** 654–666

Tao R, Coleman MC, Pennington JD, Ozden O, Park SH, Jiang H, Kim HS, Flynn CR, Hill S, McDonald WH, Olivier AK, Spitz DR & Gius D (2010) Sirt3-Mediated Deacetylation of Evolutionarily Conserved Lysine 122 Regulates MnSOD Activity in Response to Stress. *Mol. Cell.* **40:** 893-904

Thakur BK, Dittrich T, Chandra P, Becker A, Lippka Y, Selvakumar D, Klusmann JH, Reinhardt D & Welte K (2012) Inhibition of NAMPT pathway by FK866 activates the function of p53 in HEK293T cells. *Biochem. Biophys. Res. Commun.* **424:** 371-377

Todisco S, Agrimi G, Castegna A & Palmieri F (2006) Identification of the mitochondrial NAD+ transporter in Saccharomyces cerevisiae. *J. Biol. Chem.* **281:** 1524-1531

VanLinden MR, Dölle C, Pettersen IKN, Kulikova VA, Niere M, Agrimi G, Dyrstad SE, Palmieri F, Nikiforov AA, Tronstad KJ & Ziegler M (2015) Subcellular Distribution of NAD+ between Cytosol and Mitochondria Determines the Metabolic Profile of Human Cells. *J. Biol. Chem.* **290:** 27644–27659

VanLinden MR (2015). Dissertation: Insights into NAD homeostasis in the compartmentalized cell

VanLinden MR, Niere M, Nikiforov AA, Ziegler M & Dölle C (2017) Compartment-specific poly-ADP-ribose formation as a biosensor for subcellular NAD pools. *Methods Mol Biol.* **1608:** 45-56

van Roermund CW, Elgersma Y, Singh N, Wanders RJ & Tabak HF (1995) The membrane of peroxisomes in Saccharomyces cerevisiae is impermeable to NAD(H) and acetyl-CoA under in vivo conditions. *EMBO J.* **14:** 3480-3486

Vaziri H, Dessain SK, Eaton EN, Imai SI, Frye RA, Pandita TK, Guarente L & Weinberg RA (2001) hSIR2SIRT1 functions as an NAD-dependent p53 deacetylase. *Cell.* **107:** 149-159

Visser WF, van Roermund CWT, Ijlst L, Waterham HR & Wanders RJA (2007) Metabolite transport across the peroxisomal membrane. *Biochem. J.* **401:** 365-375

Vyas S, Chesarone-Cataldo M, Todorova T, Huang YH & Chang P (2013) A systematic analysis of the PARP protein family identifies new functions critical for cell physiology. *Nat. Commun.* **4:** 2240

Wanders RJA (2014) Metabolic functions of peroxisomes in health and disease. *Biochimie.* **98:** 36-44

Wanders RJA & Waterham HR (2006) Peroxisomal disorders: The single peroxisomal enzyme deficiencies. *Biochim. Biophys. Acta - Mol. Cell Res.* **1763:** 1707-1720

Wanders RJA, Waterham HR & Ferdinandusse S (2016) Metabolic Interplay between Peroxisomes and Other Subcellular Organelles Including Mitochondria and the Endoplasmic Reticulum. *Front. Cell Dev. Biol.*

Wang S, Yang X, Lin Y, Qiu X, Li H, Zhao X, Cao L, Liu X, Pang Y, Wang X & Chi Z (2013) Cellular NAD depletion and decline of SIRT1 activity play critical roles in PARP-1-mediated acute epileptic neuronal death in vitro. *Brain Res.* **1535:** 14–23

Wang Y, Zhou L, Zhao Y, Wang S, Chen L, Liu L, Ling Z, Hu F, Sun Y, Zhang J, Yang C, Yang Y, Xiong Y, Guan K & Ye D (2014) Regulation of G 6PD acetylation by KAT9 / SIRT2 modulates NADPH homeostasis and cell survival during oxidative stress. **33:** 1–17

Waterham HR, Ferdinandusse S & Wanders RJA (2016) Human disorders of peroxisome metabolism and biogenesis. *Biochim. Biophys. Acta - Mol. Cell Res.* **1863:** 922-933

Wiesinger C, Kunze M, Regelsberger G, Forss-Petter S & Berger J (2013) Impaired very long-chain acyl-CoA β-oxidation in human X-linked adrenoleukodystrophy fibroblasts is a direct consequence of ABCD1 transporter dysfunction. *J. Biol. Chem.* **288:** 19269-19279

Wood JG, Regina B, Lavu S, Hewitz K, Helfand SL, Tatar M & Sinclair D (2004) Sirtuin activators mimic caloric restriction and delay ageing in metazoans. *Nature* **430:** 686–689

Xiao Y, Kwong M, Daemen A, Belvin M, Liang X, Hatzivassiliou G & O'Brien T (2016) Metabolic response to NAD depletion across cell lines is highly variable. *PLoS One.* **11:** e0164166

Yamamoto M, Hikosaka K, Mahmood A, Tobe K, Shojaku H, Inohara H & Nakagawa T (2016) Nmnat3 is dispensable in mitochondrial NAD level maintenance in vivo. *PLoS One.* **11:** e0147037

Yang H, Yang T, Baur JA, Perez E, Matsui T, Carmona JJ, Lamming DW, Souza-Pinto NC, Bohr VA, Rosenzweig A, de Cabo R, Sauve AA & Sinclair DA (2007) Nutrient-Sensitive Mitochondrial NAD+ Levels Dictate Cell Survival. *Cell* **130:** 1095–1107

Yoshino J, Mills KF, Yoon MJ & Imai SI (2011) Nicotinamide mononucleotide, a key NAD + intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice. *Cell Metab.* **14:** 528–536

Zhang T, Berrocal JG, Frizzell KM, Gamble MJ, DuMond ME, Krishnakumar R, Yang T, Sauve AA & Lee Kraus W (2009) Enzymes in the NAD+ salvage pathway regulate SIRT1 activity at target gene promoters. *J. Biol. Chem.* **284:** 20408–20417

Zhang T & Kraus WL (2010) SIRT1-dependent regulation of chromatin and transcription: Linking NAD+ metabolism and signaling to the control of cellular functions. *Biochim. Biophys. Acta - Proteins Proteomics.* **1804:** 1666-1675

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