"Linking lipids to acetylation" novel roles of of PI(5)P and PIP4K in SIRT1 regulation and development

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Chapter 1

GENERAL INTRODUCTION

1

PREFACE

Every cell is able to communicate by sending, receiving or responding to signals in its environment. In order to trigger a physiological response, these signals must be transmitted across the cell membrane. Cells have developed a complex array of mechanisms to sense changes in the extracellular environment and to transduce these changes into intracellular signals (signal transduction) that can generate a cellular response. Signal transduction can be initiated by a ligand binding to a specific receptor at the plasma membrane. These ligands (primary messengers) bind to a diverse family of receptors, such G protein-coupled receptors, tyrosine kinase receptor or serine-threonine kinase receptors. Upon ligand binding, receptors activate diverse signaling pathways giving rise to change in abundance of intracellular signaling molecules, called second messengers. One important group of second messengers is the phosphoinositide family.

PHOSPHOINOSITIDES

Phosphoinositides are distinct class of phospholipids, consisting of an inositol head group that is linked, via a phosphate diester bond, to the 1, 2-diacylglycerol (DAG) moiety. The hydrophobic DAG moiety remains buried in the membrane (can maintain the phosphoinositide as part of the membrane), whereas the inositol head group is soluble and can interact with components (proteins) in the cytosol. Phosphoinositides can be hydrolysed by phospholipase C family enzymes to generate two important second messengers; DAG, which can stimulates protein kinase C (PKC) activity, and inositol-(1,4,5)trisphosphate (IP3), which triggers calcium release from intracellular stores (Berridge, 1987; Hirasawa, 1985). Phosphatidylinositol (PI) can also be sequentially phosphorylated at the 3', 4' and 5' positions of the inositol ring to generate seven different (biologically active) phosphoinositides, namely PI(3)P, PI(4)P, PI(5)P, PI(3,4)P_2, PI(3,5)P_2, PI(4,5)P_2 and PI(3,4,5)P_3 (Lietha, 2001). The (de)phosphorylation of phosphoinositides and its derivatives is catalyzed by distinct lipid kinases and phosphatases, each specific for a given hydroxyl or phosphate group at the inositol ring (Sasaki *et al.*, 2009). In this way, phosphoinositide-specific kinases and phosphatases can generate various bioactive phosphoinositides in specific subcellular compartments (Figure 1). This subcellular distribution is regulated by specific protein-protein interactions unique to each kinase or phosphatase (Kutateladze, 2010). This allows the generation of a spatially regulated phosphoinositide signaling system.

Phosphoinositides can regulate numerous cellular processes, including ion channel function, protein localization and enzymatic activity, which in turn can affect many downstream processes such as vesicular transport, cell polarity and migration, gene transcription, cytoskeletal dynamics and cell proliferation and survival (Di Paolo *et al.*, 2006; Irvine, 2005; McCrea *et al.*, 2009). Thus, phosphoinositides function (exert their roles) either as precursors of second messengers (DAG and IP3), or by interacting with target proteins that contain specific phosphoinositide-interacting domains (PIDs). Over the past decades, a number of protein domains have been identified that can bind to phosphoinositides with varying degrees of specificity and affinity (Figure 1). These include the pleckstrin homology domain (PH), Phox domain (PX), epsin N-terminal homology domain (ENTH), FYVE domain and lysine/arginine-rich peptide sequences (Kutateladze, 2010; Lemmon, 2008). The interaction of phosphoinositides with these domains is key to the function of many signaling proteins and networks.

NUCLEAR PHOSPHOINOSITIDES

Most of our knowledge of phosphoinositide signaling is derived from receptor-mediated signaling pathways in the cytosol, yet evidence has emerged on the existence of phosphoinositide pathways in the nucleus. Many studies have shown that nuclear phosphoinositide metabolism and its regulation are independent of their cytoplasmic/plasma membrane counterparts (Irvine, 2003). Nuclear phosphoinositides have been shown to play key roles in a wide range of nuclear events, including cell proliferation and differentiation, DNA repair, mRNA splicing but also chromatin structure and transcription as will be described in **chapter 3** of this thesis.

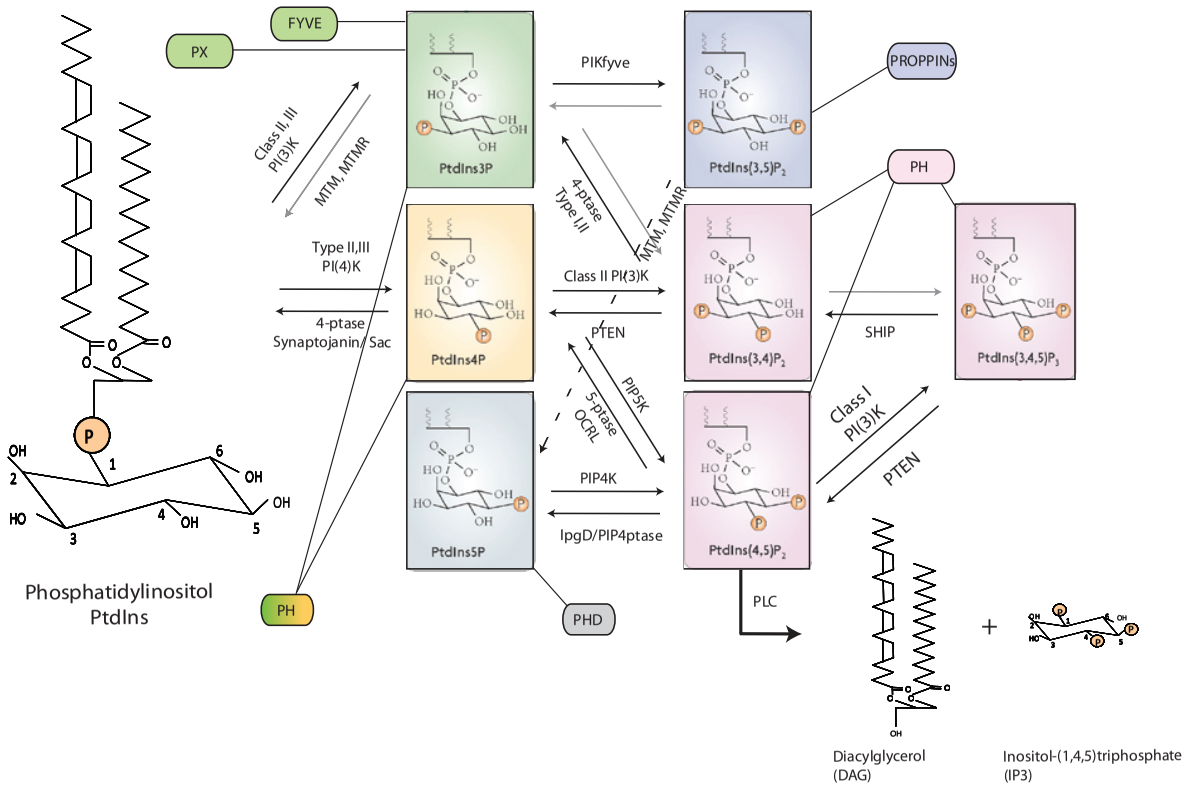

Figure 1. *Pathways of phosphoinositide synthesis and degradation. The phosphatidylinositol (PI) molecule includes inositol 1-phosphate bound via its phosphate group to 1,2-diacylglycerol (DAG) moiety. The structure and interconversion are shown for all seven known phosphoinositides namely PI(3)P, PI(4)P, PI(5)P, PI(3,4)P_2, PI(3,5)P_2, PI(4,5)P_2 and PI(3,4,5)P_2 in eukaryotes. Grey arrows represent less well-characherized pathways or pathways that have not been established yet. The phospholipid-binding domains that recognize specific phosphoinositides are indicated in different colours. PI(4,5)P_2 and other phosphoinositides can be hydrolysed by phospholipase C (PLC) enzymes to generate inositol 1,4,5-trisphosphate and diacylglycerol.*

PHOSPHATIDYLINOSITOL PHOSPHATE KINASES (PIPKS)

The generation of phosphatidylinositol 4,5-bisphosphate PI(4,5)P_2 by phosphatidylinositol phosphate kinases (PIPK) is central to phosphoinositide signaling not only in the cytosol but also within the nucleus (Bunce, 2006). PI(4,5)P2 can be synthesized by two different classes of PIPKs that utilize different substrates (van den Bout *et al.*, 2009). Phosphatidylinositol-4-phosphate 5 kinase (PIP5K) phosphorylates PI(4)P on the 5 position on the inositol ring, while phosphatidylinositol-5-phosphate 4 kinase (PIP4K) phosphorylates PI(5)P on the 4 position (Loijens *et al.*, 1996; Rameh *et al.*, 1997; Roberts *et al.*, 2005). Although PI(3)P can acts a substrate, it is much less efficiently catalyzed than PI(5)P (Morris *et al.*, 2000). Several isoforms of (consisting of α, β, γ isoforms) PIP5Ks and PIP4Ks have been found to localize in the nucleus. Despite synthesizing the same product, PIP5Ks and PIP4Ks appear functionally nonredundant. For instance, genetic deletion of PIP5K can be rescued by expression of PIP5Ks but not PIP4Ks, suggesting that PIP4Ks do not increase the cellular levels of PI(4,5)P_2 to the same extent as do the PIP5Ks. Because the cellular levels of PI(5)P is present at much lower levels than PI(4)P in the cell, the amount of PI(4,5)P_2 synthesized by PIP4K relative to PIP5K is likely to be much smaller (Clarke *et al.*, 2001; Morris *et al.*, 2000; Rameh *et al.*, 1997; Roberts *et al.*, 2005). Therefore, it is thought that PIP4Ks primarily function to remove the substrate PI(5)P and thereby regulate its potential signaling functions (as will be detailed below).

PHOSPHATIDYLINOSITOL 5-PHOSPHATE 4-KINASE (PIP4K)

The three different PIP4K isoforms, encoded by distinct genes, are found at different cellular locations. The PIP4Kα isoform is predominantly localized in the cytosol, although there is also a significant amount in the nucleus, whereas PIP4Kγ localizes to the Golgi and intracellular vesicles (Boronenkov *et al.*, 1995; Clarke *et al.*, 2008). PIP4Kβ is predominantly nuclear; its 16 amino acid α-helix insertion (not present in the other PIP4K isoforms) is essential for nuclear localization (Ciruela *et al.*, 2000). The relative expression levels of PIP4K isoforms can differ between various tissues. The ratio of PIP4Kβ compaired to PIP4Kα and PIP4Kγ expression is particularly high in skeletal muscle, where there is approximately 10-fold more PIP4Kβ than PIP4Kα and PIP4Kγ (Lamia *et al.*, 2004). Furthermore, in situ hybridation studies and RT-PCR in mice revealed similar expression of PIP4Kα and β in the brain, whereas PIP4Kα is expressed at higher levels than PIP4Kβ and γ in spleen. PIP4Kγ is particularly highly expressed in the kidney compared to PIP4Kα and β (Clarke *et al.*, 2008; Volpicelli-Daley *et al.*, 2010). These studies indicate that the differential expression and subcellular localization of each PIP4K isoform may serve specific functions (depending on cell and tissue context) of each organism (Clarke, 2010). The three isoforms of PIP4Ks also differ greatly in catalytic activity: PIP4Kα has the highest activity, followed by PIP4Kβ which has 2000-fold less activity towards PI(5)P compared to PIP4Kα, whereas PIP4Kγ is generally thought to be inactive (Bultsma *et al.*, 2010; Clarke *et al.*, 2008; Wang *et al.*, 2010). However, recent evidence indicates that distinct isoforms can affect one another's sub-cellular localization (Clarke *et al.*, 2012). For instance, the high activity PIP4Kα isoform can interact with PIP4Kβ possibly by forming a heterodimer. The interaction and targeting of different isoforms suggest a mechanism by which low activity isoforms like

PIP4Kβ can regulate PI(5)P levels in a given cellular compartment (Bultsma *et al.*, 2010). Finally, it should be mentioned that the physiological roles of PIP4Ks remain poorly understood. PIP4Kβ-deleted mice are mildly growth retarded and hypersensitive to insulin (Lamia *et al.*, 2004). However, knockout or knockdown phenotypes of PIP4Kα and PIP4Kγ have not been reported to date. **Chapter 5** examines the role of PIP4Ks in vertebrate embryonic development using zebrafish as a model.

PHOSPHATIDYLINOSITOL 5-PHOSPHATE (PI(5)P) SIGNALING

Function

PI(5)P is the last identified member of the seven known phosphoinositides. Its late discovery is probably due to the fact that PI(5)P is low abundant (1-5%) compared to PI(4)P (Rameh *et al.*, 1997; Sarkes *et al.*, 2010). Therefore, PI(5)P has long been considered to be just an intermediate metabolite in the synthesis of other phosphoinositides. PI(5)P resides in the plasma membrane, intracellular membranes and the nucleus. Interestingly, several physiological and pathological stimuli alter cellular PI(5)P levels. For instance, the levels of PI(5)P are very low in resting cells, but they rise upon thrombin and insulin stimulation in human platelets or by osmotic stress in plant cells (Meijer, 2001; Morris *et al.*, 2000; Ndamukong *et al.*, 2010; Sbrissa *et al.*, 2004). During bacterial infection with Shigella flexneri or Salmonella typhimurium, the levels of PI(5)P are elevated and were shown to facilitate infection (Niebuhr *et al.*, 2002; Terebiznik, 2002). Moreover, a nuclear pool of PI(5)P has been found to increase when cells progress through the cell cycle and in response to oxidative stress and ultraviolet (UV) irradiation (Clarke *et al.*, 2001; Jones *et al.*, 2006; Jones *et al.*, 2012; Keune *et al.*, 2012).

As described above, phosphoinositides exert their function through interaction with specific phosphoinositide-binding domains. Identification of the binding of PI(5)P to ING2 (Inhibitor of Growth Protein 2) through the plant homeobox domain (PHD) motif defined this domain as the first PI(5)P phosphoinositide-binding domain in the nucleus(Gozani *et al.*, 2003). ING2 is a candidate tumor suppressor that induces growth arrest and apoptosis in a p53-dependent manner (Nagashima, 2001; Shi, 2005). Mutations resulting in loss of interaction with PI(5)P affect localization and the activity of ING2, leading to a decrease in ING2-mediated apoptosis and p53 acetylation. Another example of PI(5)P-PHD domain interaction is the relocalization of the histone H3 lysine 4 methylase ATX1 from the nucleus to the cytoplasm in response to dehydration stress (Alvarez-Venegas *et al.*, 2006; Ndamukong *et al.*, 2010b). Furthermore, the activity of the cullin 3 (CUL3) ubiquitination complex appears to be stimulated by nuclear PI(5)P (Bunce, 2008). Together, these studies indicate that nuclear PI(5)P levels play important roles in regulating nuclear protein function, especially in nuclear events such as chromatin remodeling, as discussed in **Chapter 3**.

PI(5)P signaling functions in the cytosol have also been reported (Grainger, 2012). In the cytosol, overexpression of PIP4Kβ resulted in a decrease in Akt activity in response to insulin (Carricaburu, 2003). Conversely, cells expressing IpgD, a bacterial PI(4,5)P_2-4-phosphatase that generates PI(5)P, had higher levels of basal and insulin-stimulated Akt phosphorylation (Pendaries, 2006). Interestingly, deletion of PIP4Kβ in mice also leads to increased insulin-induced Akt activation in muscle (Lamia *et al.*, 2004). Additionally, it has recently been shown

that PI(5)P-dependent Akt activation occurs via translocation of the EGF receptor (Ramel *et al.*, 2011). Next to insulin, H_2O_2-induced PI(5)P levels also regulate the activity of Akt, which in turn is important for cell growth inhibition by H_2O_2(Jones *et al.*, 2012). Furthermore, PI(5)P has been shown to play a role in cell morphology by mediating actin rearrangements downstream of the insulin receptor (Sbrissa *et al.*, 2004). More recently, PI(5)P was shown to promote cell migration through the activity of specific kinases and phosphatases involved in metabolizing PI(3,5)P_2 (Oppelt *et al.*, 2012).

Regulation

How is the abundance of PI(5)P regulated in distinct cellular compartments? Several pathways have the potential to generate PI(5)P, but their relative contributions to PI(5)P regulation *in vivo* are unclear (Figure 2). PI(3)P and PI(4)P phosphoinositides are produced by phosphorylation of PI by their specific kinases; however, no such kinase has been identified for PI(5)P so far (Lecompte *et al.*, 2008). Instead, it appears that PI(5)P production is regulated by specific phosphatases that dephosphorylate PI(4,5)P_2. This pathway was first identified by

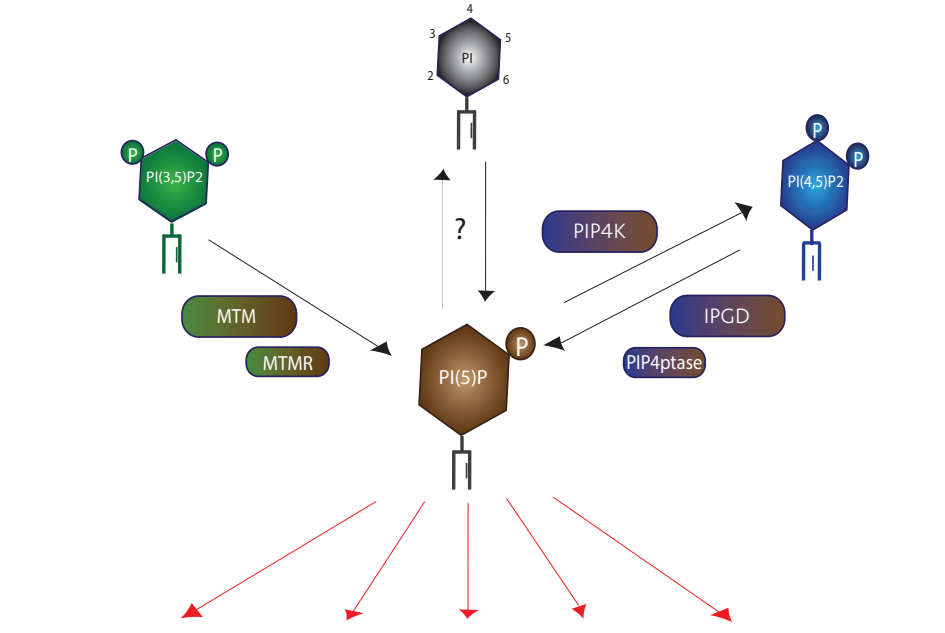

Figure 2. *Regulation of PI(5)P and its distinct roles in nuclear and cytosolic signaling. PI(5)P can be synthesized by MTM/MTMR phosphatases via dephosphorylation of PI(3,5)P_2 and bacterial IpgD and mammalian Type I,II PIP-4-phosphatases that convert PI(4,5)P_2 to PI(4,5)P_2. PIP4K can phosphorylate PI(5)P to PI(4,5)P_2 which is important for the physiological regulation of PI(5)P and downstream signaling. PI(5)P binds to PHD domain present on ING2, ATX1 and other nuclear proteins involved in nuclear signaling. Furthermore PI(5)P levels plays an important role in other cellular functions such as cell migration, insulin responses etc.*

the PI(4,5)P_2 -4-phosphatase IpgD, responsible for the profound increase in PI(5)P observed in S.Flexneri-infected cells (Niebuhr *et al.*, 2002). Subsequently, two human homologous PI(4,5)P_2 -4-phosphatases have been characterized that can translocate to the nucleus upon stress induction (Zou, 2007). The abundance of PI(5)P can also be increased by the stress-activated lipid kinase PIKfyve, whose main function is to produce PI(3,5)P_2 from PI(3)P (Tolias *et al.*, 1998). Overexpression of PIKfyve increases cellular PI(5)P (Sbrissa, 2002). Mice lacking one copy of the gene have reduced PI(5)P levels (Ikonomov, 2011). However, PI(5)P can also be generated by the major product of PIKfyve, PI(3,5)P_2, by myotubularin 3-phosphatases(Schaletzky, 2003). For example, MTM1 overexpression is required for enhanced PI(5)P production in osmotically stressed muscle (Tronchere *et al.*, 2004). However, the ability of myotubularin to dephosphorylate PI(5)P and their operation *in vivo* is not yet established.

Unlike the dephosphorylation to PI(5)P, the PI(5)P removal via PI(5)P phosphorylation is much better established. PIP4Ks plays an important role in the regulation of PI(5)P levels in the nucleus, where stressing cells activates p38 MAP kinase, which phosphorylates PIP4Kβ on Ser^{326} (Jones *et al.*, 2006). This decreases its PIP4K activity leading to an increase in the nuclear abundance of PI(5)P. A possible explanation of how PIP4Kβ, (which has low catalytic activity) can regulate nuclear PI(5)P levels comes from the observation that PIP4Kα and PIP4Kβ can form heterodimers (Bultsma *et al.*, 2010). Thereby PIP4Kβ can target PIP4Kα activity to the nucleus. How phosphorylation by the p38 pathway regulates the PIP4Kα/PIP4Kβ complex and its activity is currently not clear. PIP4Kβ is heavily post-translationally modified: in addition to being phosphorylated, it is also extensively acetylated. Consequently, these modifications might regulate PIP4Kα/PIP4Kβ complex formation. In **Chapter 4**, we describe acetylated residues on PIP4Kβ and we identify it as a target of the SIRT1 deacetylase (see below). In conclusion, the metabolic pathways governing the levels and the signaling roles of PI(5)P are still not well understood, but recent data reveal its function as lipid mediator.

PROTEIN ACETYLATION

Acetylation refers to the addition of an acetyl group on lysine residues present within both histone and non-histone proteins. Acetylation is mediated by histone acetyl transferases (HATs). Since a large number of nonhistone proteins are targeted by HATs, these enzymes are also called K-acetyltransferases (KATs) (Berndsen, 2008). Acetylation of histones directly influences chromatin remodeling, by reducing the positive charge on lysine residues and by decreasing the affinity of histones for negatively charged DNA. This allows a localized "unraveling" of chromatin, making it more accessible for the binding of coactivators and the basal transcription machinery (Jiang, 2009).

The removal of acetyl groups is catalyzed by histone deacetylases (HDACs) and is associated with chromatin condensation and transcriptional repression. HDACs exert their repressive function on transcription either by condensing the chromatin or as components of large multiprotein complexes, by recruiting inhibitory factors to gene promoter regions (Jiang, 2009). Transcriptional regulation exerted by HDACs determines many cellular processes including cell cycle progression, apoptosis, autophagy, response to diverse types of stress, differentiation, and development (Norris *et al.*, 2009). Mammalian HDACs can be classified

into one of four different classes based on their amino acid sequence and structure. Sirtuins, or class III HDACs, have no homology to class I, II, or IV HDACs (TSA-sensitive enzymes and are localized in both the nucleus and the cytoplasm). Their deacetylase activity depends on the cofactor NAD^+, rather than zinc, and they are localized in the nucleus, cytoplasm, or mitochondria and can deacetylate histone and nonhistone proteins (Imai *et al.*, 2000).

SIRTUINS

Sirtuins are highly conserved from bacteria to human and SIR2 (silent mating-type information regulation 2) was originally shown to extend lifespan in budding yeast (Kaeberlein, 1999). In mammals, there are seven sirtuins (SIRT1-7) all containing a conserved NAD-binding and catalytic domain, termed the sirtuin core domain, but they differ in their N and C-terminal domains (Frye, 2000). Sirtuins show significant sequence and functional differences from other classes of HDACs in that they carry out deacetylation via a two-step reaction that consumes NAD^+ and releases, O-acetyl-ADP-ribose (AADPR), the deacetylated substrate and nicotinamide (used as sirtuin inhibitor) (Houtkooper, 2010). Sirtuins, although relatively similar to each other, have divergent biological functions which can be due to distinct cell-type-specific subcellular localisation of each member of the family (Finkel *et al.*, 2009). SIRT1 is located in both the nucleus and the cytoplasm, SIRT2 in the cytoplasm, SIRT3, 4, and 5 are mitochondrial, and SIRT6 and 7 are nuclear (Michan *et al.*, 2007). Apart from intracellular localization, SIRT1, 3, and 5 differ from SIRT2, 4, and 6 in the type of reaction they catalyse. SIRT1, 3, and 5 are NAD^+-dependent deacetylases catalyzing the deacetylation of histones and nonhistone proteins, whereas SIRT6 is a NAD^+-dependent ADP ribosyltransferase (ART) mediating mitochondrial protein ribosylation; SIRT2 and 4 exert both NAD^+-dependent HDAC and ART activities (Westphal, 2007). The enzymatic activity of SIRT7 as well as its specific substrates has not yet been determined.

SIRT1 FUNCTIONS

SIRT1 is the most evolutionarily conserved sirtuin among the seven mammalian homologs, and it has been shown to play crucial roles in complex physiological processes, including metabolism, cancer, and aging (Sebastian *et al.*, 2012). SIRT1 participates in various cellular functions ranging from differentiation and development to metabolism and cell survival by deacetylating diverse substrates, summarized in Figure 3 (Nakagawa *et al.*, 2011). SIRT1 can deacetylate histone H4K16, H3K9 and H1K26 and thereby mediate heterochromatin formation (Liu *et al.*, 2013). Through its enzymatic activity, SIRT1 has also the capacity to regulate the activity of various transcription factors and other regulatory proteins. For example, SIRT1 regulates energy by inducing gluconeogenic while repressing glycolytic gene expressions through deacetylation of PGC-1α and PPARγ (Picard, 2004; Rodgers, 2005). Deacetylation of NFKB, AP1 and Foxp3 by SIRT1 modulates the inflammation and immune pathway (Kong, 2012). And the deacetylation of DNA repair proteins Ku70, NBS1, WRN and XPA by SIRT1 regulates genomic stability (Fan, 2010; Li *et al.*, 2008; Yuan, 2007). SIRT1 regulates cell growth, apoptosis and stress response by deacetylating tumor suppressor protein like forkhead box protein FOXO1,3 and 4, Hif-1α, HSF1, Rb and survivin (Brunet *et al.*, 2004; Lim, 2010; Luo, 2008; Motta *et al.*, 2004; Wang *et al.*, 2008;

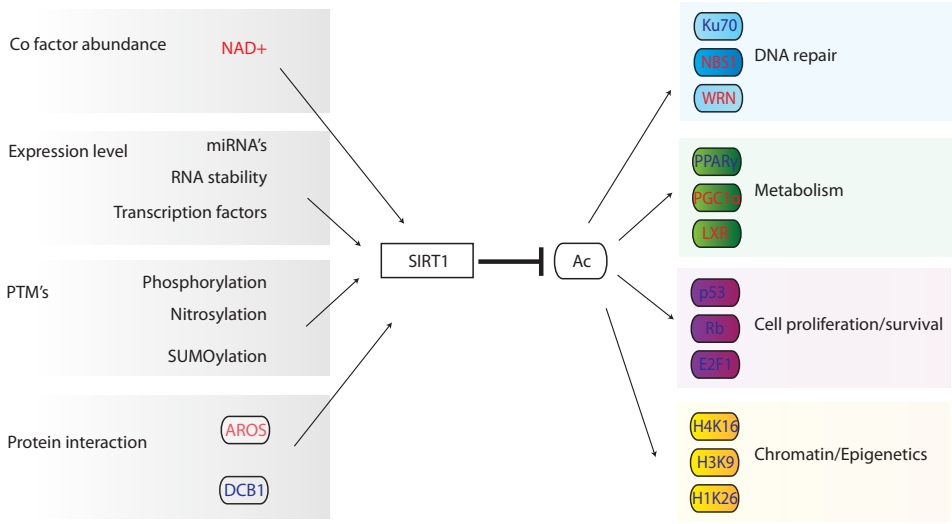

Figure 3. *SIRT1 pathway overview. SIRT1 is an NAD⁺-dependent histone deacetylase that catalyses the removal of acetyl (Ac) groups from a number of non-histone targets. The downstream effects of target deacetylation include changes in chromatin structure, cellular metabolism as well as cell survival and DNA repair. Several factors are involved in the regulation of SIRT1 function. SIRT1 function can be controlled availability of NAD⁺, posttranslational modifications, changes in expression or protein-protein interactions.*

Wong, 2007). The first and most widely known substrate of SIRT1 is tumor suppressor p53, which has critical roles in the cell-cycle regulation and apoptosis(Luo *et al.*, 2001; McCubrey, 2012).

p53 is a short-lived protein that is sustained at low levels under normal physiological conditions. However, p53 is maintained at a relative high level by posttranslational modifications in response to various stresses (Gu, 2012). The acetylation of p53 in response to DNA damage by HAT CBP/p300 was shown to acetylate p53 at K373/K382 and K320. Lysine acetylation at these sites promotes both its DNA binding ability and its transcriptional activity, and include p21, Bax and puma as p53 targets (Smith, 2002). In mice harboring a mutant p53 allele in which all the acetylation sites have been replaced a deficiency in gene transcription in response to DNA damage was shown, which illustrates that acetylation is indispensible for p53 function (Chao *et al.*, 2006; Tang *et al.*, 2008). Conversely, SIRT1 binds to and deacetylates p53, thereby negatively regulating p53-mediated transcriptional activation. SIRT1 functions by deacetylating of p53 which prevents cellular senescence and apoptosis caused by DNA damage and stress (Luo *et al.*, 2001; Vaziri *et al.*, 2001).

SIRT1 REGULATION

Although much attention has been focused on the identification of the cellular targets and functional networks controlled by SIRT1, the mechanisms that regulate SIRT1 activity by biological stimuli have only recently begun to emerge. As an enzyme, the activity of SIRT1 can

be controlled by the availability of its substrates, post-translational modifications, interactions with other proteins and changes in its expression levels (Figure 3) (Revollo *et al.*, 2013).

The basal intracellular NAD^+ levels are maintained relatively constant by the NAD^+ biosynthetic and salvage pathways (Houtkooper, 2010). Proper functioning of these pathways is important for the activation of SIRT1 catalytic activity since the availability of NAD^+ in cells is a limiting step(Revollo *et al.*, 2004). Glucose deprivation and metabolic changes by forexample calorie restriction can cause fluctuations in NAD^+ levels. Since SIRT1 associate with chromatin and its function is NAD^+-dependent, SIRT1 can couple changes in the cellular metabolic state and NAD^+ levels to transcription (Chalkiadaki, 2012).

Furthermore, SIRT1 enzymatic activity can be regulated by several posttranslational modifications. First, prosurvival dual specificity tyrosine phosphorylation-regulated kinases (DYRKs), DYRK1A and DYRK3, have been shown to phosphorylate SIRT1 in response to genotoxic stress (Guo, 2010). This PTM substantially enhances SIRT1 deacetylase activity towards acetylated p53 and protects cells from genotoxic stress-induced apoptosis (Guo, 2012). Another serine residue located at the highly conserved core domain of SIRT1, Ser 434, has been shown to be a phosphorylation target of the cyclic AMP/protein kinase A (cAMP/PKA) signaling pathway (Gerhart-Hines *et al.*, 2011). This PTM rapidly enhances the intrinsic deacetylase activity of SIRT1 independently of cellular NAD+ levels. Finally, C-Jun N-terminal kinase (JNK)1, phosphorylates SIRT1 on several serines, particularly under stressful cellular conditions (Nasrin, 2009). Intriguingly, these phosphorylations appear to increase the deacetylase activity of SIRT1 towards one of its substrates, histone H3, but have no effect towards another substrate, p53. This suggests that some or all of these PTMs can alter the activity of SIRT1 in a substrate-specific manner. SIRT1 is also sumoylated at K734, which in turn increases its activity and nitrosylation at Cys 387 and 390 in the catalytic core reduces SIRT1's ability to deacetylate PGC-1α (Kornberg, 2010; Yang, 2007).

In addition to posttranslational modification, alteration in expression levels can also regulate SIRT1 activity. The "tumor suppressor hypermethylated in cancer" protein (HIC1) has been shown to inhibit SIRT1 expression by forming a repressive complex with SIRT1 on its own promoter (Chen, 2005; Zhang *et al.*, 2007). Conversely, through a DNA damage dependent matter transcription factor E2F1 can induce SIRT1 expression (Wang *et al.*, 2006). Members of the FOXO transcription factors also regulated the expression of SIRT1, like FOXO1, which induces SIRT1 expression by binding to FOXO1 response elements in the SIRT1 promoter (Nemoto, 2004; Xiong, 2011). The SIRT1 promoter also contains several cyclic AMP response element-binding protein (CREB) binding sites and through CREB, a transcription factor whose activation is mediated by PKA in response to low nutrient availability, the expression of SIRT1 is induced (Fusco, 2012; Noriega, 2011). The abundance of SIRT1 is also controlled by translational events and RNA stability. For instance, the Hu antigen (HuR) an mRNA binding protein that binds the 3'UTR of SIRT1 mRNA and plays a major role in stabilizing SIRT1 mRNA transcript (Abdelmohsen, 2007; Yamakuchi, 2012). Furthermore, SIRT1 expression and its activity are also under the influence of several miRNA's as reported by several studies.

Finally, protein–protein interactions also play key roles in the regulation of SIRT1. For instance, interaction with active regulator of SIRT1 (AROS) enhances the activity of SIRT1 towards acetylated p53. AROS appears to bind to the N terminus of SIRT1, but little is known

about how this interaction increases the SIRT1 activity (Kim *et al.*, 2007). In contrast to AROS, deleted in breast cancer 1 (DBC1) is a negative regulator of SIRT1 (Kim *et al.*, 2008; Zhao *et al.*, 2008). The 'essential for SIRT1 activity' (ESA) domain (a 25-amino-acid region in the C terminus of SIRT1), which intramolecularly interacts with the SIRT1 deacetylase core, was also shown to activate its enzymatic activity (Kang *et al.*, 2011). DBC1 also interacts with the SIRT1 deacetylase core, potentially displacing ESA, decreasing the accessibility of the core domain to protein substrates, and thereby inactivating SIRT1. Moreover, cellular stress results in phosphorylation of DBC1 at Thr 454, which appears to create a second binding site for SIRT1 to augment the DBC1–SIRT1 interaction (Yuan, 2012). However, for most of studies where SIRT1 activity was shown to be modulated by protein-protein interactions, it is not clear yet whether allosteric changes or substrate access to catalytic core are responsible for the decrease in SIRT1 activity.

These studies show that SIRT1 is temporally and spatially regulated in response to various environmental cues. In **Chapter 2**, we describe a novel aspect of the regulation of SIRT1 by its interaction with and activation by phosphoinositides.

THESIS OUTLINE

The studies described in this thesis focus on the novel role of the PI(5)P/PIP4K signaling axis in regulating acetylation and understanding their physiological functions. **Chapter 1** is a general introduction giving an overview of the molecules and pathways involved in phosphoinositide signaling and regulation of SIRT1 activity. **Chapter 2** identifies PI(5)P as novel lipid regulator of SIRT1 activity and that SIRT1 is subject to acute regulation in response to lipid signaling. **Chapter 3** reviews the latest insights of phospholipids in the nucleus and their emerging role as important modulators of chromatin and gene transcription. Next, **chapter 4** explores the posttranslational modifications on PIP4Kβ and describes its biochemical interaction and deacetylation by SIRT1 deacetylase**. Chapter 5** provides insight on the physiological roles of PIP4Ks and provides the first description of how lack of PIP4Kα expression and activity affects zebrafish development. In **chapter 6**, the results presented in this thesis are summarized and discussed

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