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Rerouting 'coenzyme A' biosynthesis

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CHAPTER 6

Summarizing discussion and conclusion

Adopted and modified from:
Article - Coenzyme A, more than 'just' a metabolic cofactor
Authors - Balaji Srinivasan and Ody C.M.Sibon
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CONTENTS

COENZYME A IS MORE THAN 'JUST' A METABOLIC COFACTOR

In all organisms, there are various metabolites that play a vital role to enable proper cellular metabolic processes. The human metabolome comprises a total of more than 25,000 identified endogenous metabolites[1]. Such biological molecules or metabolites, mainly vitamins, amino acids, lipids, carbohydrates, nucleic acids, and nucleotides, are involved in important biological pathways and metabolic circuits[1-3]. Alteration of metabolite homeostasis disrupts the physiology of cells, leading to various diseases[2]. Recent studies advance our understanding that some metabolites are not only involved in cellular metabolism, but also have other molecular functions. It has become evident that similar to multifunctional 'moonlighting proteins', 'moonlighting metabolites' also exists. For example, nicotinamide adenine dinucleotide (-NAD) was shown to regulate metabolism and also sirtuin deacetylase activity. This direct dual role of NAD could not be anticipated initially[4,5]. Recent findings now also indicate that CoA, another metabolic cofactor, can be considered as being more than 'just' a metabolic cofactor, and altered CoA levels lead to severe and complex effects.

The importance of CoA and acyl-CoA in essential metabolic pathways like Kreb's cycle and fatty acid β-oxidation was previously well-studied[6.] However, the role of acetyl-CoA in protein post-translational modification emerged more recently as an interesting focus in biological sciences[7]. Cellular citrate and pyruvate levels are primarily considered to regulate acetyl-CoA levels[7,8]. Whereas the CoA biosynthesis pathway is crucial for the maintenance of free CoA levels, which subsequently can be converted into acetyl-CoA and thereby can serve, as an acetyl source. In addition to the influence of CoA levels on metabolism and protein-acetylation levels, recently the metabolic cofactor CoA has shown to influence other cellular functions as well. It was demonstrated that CoA can bind directly to calcium/calmodulin-dependent protein kinase II (CaMKII) and thereby activates CAMKII in *Xenopus leavis* oocytes promoting oocyte survival via phosphorylation and inactivation of caspase-2[9]. Moreover, evidence is presented that this is a conserved mechanism which is also present in mammalian oocytes[9]. These data reveal a signaling function of CoA independent from its role in cellular metabolism (Figure 1).

Based on the initial discoveries and the recent evidence of diverse CoA regulated cellular processes, it is evident that the maintenance of proper cellular CoA biosynthesis is vital. However, there is a lack of detailed research studies to understand the consequences of impaired *de novo* CoA biosynthesis. Here in this chapter, we will address the important findings presented in this thesis about consequences of impairment of the CoA biosynthesis pathway and also we will discuss how this is related to the pathophysiology of Pantothenate Kinase-Associated Neurodegeneration.

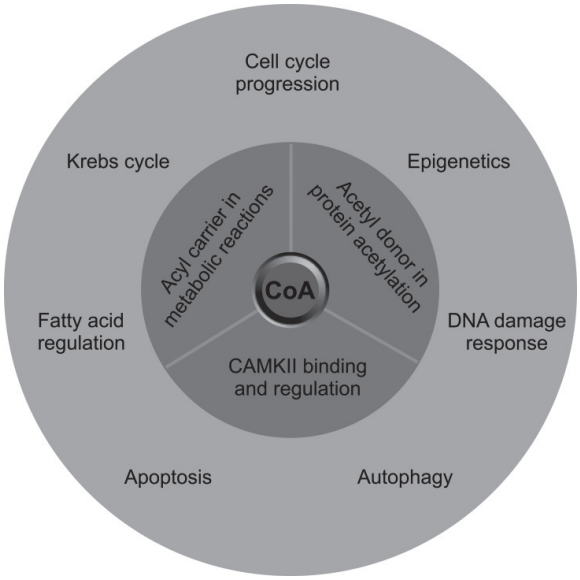

Figure 1: Schematic representation of diverse roles of CoA in cellular physiology.

Coenzyme A biosynthesis pathway and neurodegenerative diseases

The *de novo* CoA biosynthesis is carried out by the action of specific enzymes in several steps starting from pantothenate[10]. Pantothenate is present in sufficient quantities in the diet, and bacteria in human intestines also produce it. The enzymatic steps of the canonical CoA *de novo* biosynthesis route are well conserved and genes encoding for these enzymes have been identified in various organisms[11-16]. CoA regained new attention after the discovery that one of the CoA biosynthetic enzymes 'pantothenate kinase' (or PANK) was found to cause a neurodegenerative disease characterized with very specific features. After the discovery of the causative gene 'pantothenate kinase 2' in 2001, the disease was named Pantothenate Kinase-Associated Neurodegeneration (PKAN)[17].

PKAN is a subform of Neurodegeneration with Brain Iron Accumulation (NBIA), which is a group of disorders all characterized by iron accumulation in the brain[18]. Mutations in several seemingly unrelated genes (including pantothenate kinase 2) leads to a specific subform of NBIA[19,20]. PKAN patients show the clinical manifestation of a progressive loss of extrapyramidal and pyramidal functions, and patients also often suffer from dystonia, dysarthria, and intellectual disabilities[21]. The identification of the causative gene for PKAN indicated a clear link between neurodegeneration and CoA *de novo* biosynthesis and suggests that the intact homeostasis of CoA levels is highly essential for normal brain function. However, to date, the pathophysiology of this disease is largely unknown, a therapy is lacking and how impaired *de novo* biosynthesis of CoA leads to a specific subform of NBIA is still unclear.

Drosophila to understand the link between Coenzyme A and neurodegeneration

In order to investigate functions of CoA and consequences of impaired *de novo* CoA biosynthesis, several model organisms have been used, such as *Arabidopsis thaliana*[22], mice[23,24], *Schizosaccharomyces pombe*[25] and *Drosophila melanogaster*[11,14,26]. Here we will focus on the multicellular eukaryote model organism *Drosophila melanogaster* (fruit fly) and its potential aid in understanding the link between CoA and neurodegeneration will be summarized and discussed.

In 2001, a few months before PANK2 was identified as the causative gene for PKAN, Wasserman and coworkers described the phenotype of *Drosophila melanogaster* carrying mutations in the gene *fumble,* coding for pantothenate kinase[11]. The *fumble* gene is also referred to as *dPANK/fbl* (*Drosophila pantothenate kinase/fumble*)[11]. Although the researchers focused in this study on the male sterile phenotype, they also noticed the locomotor impairment of the mutant flies. Subsequently, they named the gene and the mutant after this locomotive phenotype; '*fumble*'. Wasserman and co-workers, therefore, were the first who revealed the link between impaired pantothenate kinase function and locomotor abnormalities. In addition to the locomotor defects, in *dPANK/fbl* mutant flies a decreased number of mitotic neuroblasts and an increase in abnormal (lagging chromosomes and anaphase bridges) mitotic chromosomes were observed[11]. In addition, spermatocytes of *dPANK/fbl* mutant flies also showed defects in cytokinesis and chromosome segregation. Nevertheless, why mutations in *dPANK/fbl* lead to such specific phenotypes is not known yet. Later in the same year, the Hayflick laboratory identified human PANK2 as a causative gene for the severe autosomal recessive neurodegenerative disorder PKAN, further establishing a link between impaired CoA biosynthesis and abnormal locomotor functions[17]. However, PKAN is still surrounded by unresolved basic questions, such as: why do patients with mutations in PANK2 suffer from neurological symptoms, what are the mechanisms behind the specific symptoms and why is especially the nervous system affected?

Drosophila melanogaster is a versatile and genetically manipulatable model organism. It is widely used to study basic mechanisms in life sciences at various levels (molecular, cellular, tissue, developmental and behavioral) and it is also used as a model to study human diseases[27-29]. Similarly to many diseases with a genetic cause, a *Drosophila* PKAN model was explored, and *dPANK/fbl* mutants were further investigated to understand the link between neurodegeneration and CoA biosynthesis. Firstly, the *Drosophila* genes coding for enzymes required for the *de novo* biosynthesis of CoA were annotated by *in-silico* analysis and 5 *Drosophila* loci were identified coding for structural orthologs of *PANK (dPANK/fbl), PPCS (dPPCS), PPCDC (dPPCDC)* and a bifunctional *PPAT-DPCK* (*dPPAT-DCPK;* also called *dCOASY*) and a single *dDPCK*[14]. Moreover, it has been shown that *Drosophila* mutants carrying mutations in *dPANK/fbl*, *dPPCS* and *dPPAT-DCPK* show a comparable phenotype, strongly

suggesting that impaired CoA biosynthesis causes this shared phenotype[14]. All mutants show abnormal locomotor function (impaired climbing and flying behavior) which worsens upon ageing, a decreased life span, abnormal lipid homeostasis, increased sensitivity to Reactive Oxygen Species (ROS) inducing agents, increased apoptotic cells in the brain and increased sensitivity to DNA damaging agents[14]. Moreover, consistent with the previous results from Wasserman and co-workers[11] all investigated *Drosophila* CoA mutants show increased number of cells with abnormal mitotic chromosomes in larval brains. The phenotypes of all *Drosophila* CoA mutants were comparable. However, the severity of the phenotypes varied between the mutants, most likely because the mutants are hypomorphs, and, therefore, the rest-activity of each affected enzyme may be different.

The use of *Drosophila dPANK/fbl* mutant made it possible for us to investigate whether the impaired function of *dPANK/fbl* actually leads to decreased levels of CoA. If so, this would suggest that decreased levels of the end product CoA is the primary cause of the reported defects. To investigate whether CoA biosynthesis is affected, we first measured CoA levels in both *dPANK/fbl* fly mutants and also in *dPANK/fbl* (by RNA-interference) depleted *Drosophila* cultured cells. The results presented in this thesis evidently shows that impaired dPANK/fbl activity leads to decreased levels of total CoA (Chapter 2)[30]. More interestingly, in a selective compound screening, pantethine was found to replenish the decreased CoA levels and thereby partly rescued life span, neurodegeneration and locomotor defects in *dPANK/fbl* mutant flies (Chapter 2)[30]. These data were the first to suggest that decreased levels of CoA may be causative of the observed neurodegenerative symptoms using a eukaryotic PKAN model system. These results also suggested that patients who carry mutations in other CoA biosynthesis enzymes may also suffer from a comparable disorder like PKAN. Indeed, the recent finding that the mutation in the gene coding for the last enzyme in the CoA biosynthesis pathway, PPAT-DPCK or Coenzyme A synthase (COASY), causes a new form of NBIA, referred to as CoPAN, further indicated that impairment of CoA homeostasis is strongly associated with neurodegeneration[31]. More detailed research needs to be performed to investigate whether the affected brain regions in PKAN and CoPAN show decreased CoA levels. Like vitamin-B5, Pantethine is also a known substrate for pantothenate kinase. Nevertheless, the restoration of decreased CoA levels in dPANK/fbl mutants with pantethine indicated a possible PANK-independent alternative route parallel to the canonical *de novo* CoA biosynthesis route (Chapter 2; Figure 2)[30]. Moreover, vitamin-B5 did not rescue the dPANK/fbl mutant phenotype. These results indicate the presence of an unknown kinase able to phosphorylate pantetheine, or suggests the possibility that the residual PANK enzyme present in the hypomorph dPANK/fbl mutants is having a high affinity to pantethine. More detailed research is therefore needed to understand the working mechanism of pantethine.

PKAN and CoPAN in relation to mitochondrial integrity

The next question that arises is: how do decreased levels of CoA lead to neurodegeneration? It is yet unresolved whether decreased levels of CoA only cause metabolic defects or also lead to defects in non-metabolic processes in cells. Studies in mice showed that decreased levels of CoA influence the metabolic state of an organism[23,32-35]. Therefore, the most likely underlying cause of PKAN and CoPAN is speculated to be abnormal metabolic reactions. The link between impaired cellular metabolism, mitochondrial dysfunction and severity of many neurodegenerative diseases is broadly recognized[36-38]. However, the debate of whether mitochondrial dysfunction is a primary cause or consequence of neurodegeneration remains. PKAN and CoPAN share similarities regarding mitochondria. The mitochondrial localization of both PANK2 and COASY suggests that mutations in PANK2 and COASY, lead to impaired mitochondrial function, and this may lead in turn to neurodegeneration[31,39,40]. Our research data listed in this thesis also revealed that in both dPANK/fbl mutant flies and also in dPANK/fbl depleted cultured *Drosophila* cells, mitochondria are severely affected (chapter 2)[30]. Moreover, it was also demonstrated that PANK2 downregulation in the human cell line (HEK293), resulted in impaired mitochondrial activity (chapter 2)[30]. These results are in-line with the recent studies performed in mice. HoPan treatment induced both CoA depletion and severely swollen mitochondria in the mice liver[35]. Moreover, in neurons derived from PANK2 KO mice, swollen mitochondria and defects in mitochondrial membrane potential were reported[41]. Concisely, all the above information strongly corroborated the link between PKAN and mitochondrial dysfunction. However, whether such a link also exist in CoPAN needs yet to be verified. Moreover, the impairment in CoA biosynthesis can also affect other functions independent of mitochondrial dysfunction.

Coenzyme A and its influence on protein-acetylation levels

The influence of altered CoA homeostasis on other than metabolic reactions became evident by a series of experiments, demonstrating that acetyl-CoA levels influenced protein acetylation. Acetyl-CoA provides the acetyl group, which is necessary for acetylation of proteins mediated by acetyltransferases (HAT/KAT's). Protein-acetylation is a post-translational modification influencing protein functions to a large extend[42,43]. In Saccharomyces cerevisiae or budding yeast, it is reported that acetyl-CoA synthetase mutants have decreased levels of histone acetylation[44]. Reduced levels of acetyl-CoA evoked by inactivation of the budding yeast ortholog of AMP-activated protein kinase (AMPK) also resulted in decreased histone acetylation, reduced fitness and stress resistance[45]. In addition, two recent studies also showed a link between acetyl-CoA and autophagy. In budding yeast, it was demonstrated that acetyl-CoA is a suppressor of autophagy and down-regulation of acetyl-CoA synthetase in flies enhanced autophagy and increased life span[46]. The second manuscript showed in mice and human cells that starvation resulted in decreased levels of acetyl-CoA, a reduction of acetylation of cytoplasmic proteins and induction of autophagy[47]. When acetyl-CoA

levels were manipulated under these conditions and kept at a higher level, autophagy was suppressed. This suppression of autophagy was dependent on EP300 acyltransferase activity, suggesting acetyl-CoA levels regulate EP300 activity[47]. Acetyl-CoA levels can also influence cell cycle progression because, in budding yeast, it was demonstrated that acetyl-CoA promotes acetylation of histones in the regulatory domain of the G1 cyclin CLN3 and cell cycle progression is promoted[48]. Acetyl-CoA levels are not the only influencing factor for protein acetylation, as manipulating CoA levels itself can also regulate protein acetylation levels. We showed evidence that the decrease in CoA levels in both *Drosophila in vitro* and *in vivo* PANK model systems are associated with decreased levels of histone and tubulin acetylation. Moreover, restoration of CoA levels with pantethine reverted this phenotype (chapter 3; Figure 2)[49]. Together these data indicated a strong link between CoA and/or acetyl-CoA in regulating acetylation of specific proteins.

Link between decreased histone and tubulin acetylation and neurodegeneration

Decreased histone and tubulin acetylation levels are associated with neurodegenerative diseases, and, therefore, the decreased levels of protein acetylation may explain part of the neurodegenerative phenotype of *dPANK/fbl* mutant flies. There is a strong link between cognitive function, motor neuron function, and histone acetylation[50,51]. Fewer but nonetheless compelling studies reported the association between tubulin acetylation and neuronal function. Tubulin hyperacetylation protects against axonal degeneration[52] and tubulin acetylation is required for the touch response in *C.elegans* (chapter 3)[49]. In APPPS1-21 mice (a mouse model for Alzheimer's disease), levels of acetylated tubulin are decreased and restored in the presence of knockdown of the deacetylase Hdac6. In these APPPS1-21-Hdac6-/- mice improvement of associative and spatial memory is observed compared to APPPS1-21 mice[53].

These data showed that the proper neuronal function requires normal levels of tubulin and histone acetylation. Therefore, the decreased levels of histone and tubulin acetylation, as observed in the dPANK/fbl mutants, could partly explain the neurodegenerative phenotype. The addition of valproic acid restored the decreased histone and tubulin acetylation levels and partly restored the locomotor function of mutant *dPANK/fbl* larvae, suggesting that impaired acetylation also underlies the observed phenotypes (chapter 3)[49]. *dPANK/fbl* mutants also show increased sensitivity to DNA damaging and increased accumulation of DNA damage[14]. The impairment in histone acetylation could also explain such increased sensitivity, as acetylation of histones regulates DNA damage responses in various organisms[43,54-58]. In dPANK/fbl depleted cultured *Drosophila* cells, after ionizing radiation, kinetics of histone acetylation are abnormal compared to control cells, which coincided with decreased survival of the cells after ionizing radiation. TSA rescued the decrease in protein acetylation and rescued partly the sensitivity (chapter 3)[49]. Therefore, it may be possible that part of the neurodegenerative phenotype

observed in *Drosophila* CoA mutants is because of defects in DNA damage repair pathways, causing accumulation of damaged DNA in the brain. Impaired DNA damage repair is associated with neurodegeneration and several human diseases such as Ataxia Telangiectasia (ATM), AT-like Disorder (AT-LD), Nijmegen Breakage syndrome (NBS) and others[59,60]. Although it is tempting to hypothesize that accumulation of damaged DNA in non-dividing neuronal cells leads to cell death and tissue loss, this order of events has not been convincingly demonstrated and how DNA damage leads to neurodegeneration is an unresolved puzzle[61].

Alternative source for Coenzyme A biosynthesis and its therapeutic use for PKAN

In contrast to vitamin-B5, pantethine has a prompt rescue potency in restoring CoA levels and reverting acetylation defects in PKAN model systems (Chapter 2 and 3). Moreover, pantethine is reported to revert some of the disease phenotypes in other neurodegenerative disease models, like in Parkinson disease[62,63]. However, the possible clinical use of pantethine as a rescuing molecule in patients may be limited because pantetheinases or vanins hydrolyse pantethine rapidly into cysteamine and pantothenate[64-66]. Pantetheinases are present ubiquitously in humans, especially in the intestine and are also excreted in their active form in the circulation. Although pantethine treatment has been shown to have beneficial effects in PANK2 KO mice reversing the disease phenotype induced by ketogenic diet, the rescue effect is thought to be mainly due to the anti-oxidant role of cysteamine[67]. In contrast to pantethine, cysteamine did not revert the phenotype in the *Drosophila* PKAN model system (unpublished data). To improve pantethine stability and thereby improve its applicability in humans as a rescue agent for PKAN, we synthesized a novel pantetheine derivative with a lipophilic thiobutyl triphenylphosphonium (TBTP) cation (chapter 4; Figure 2). Moreover, the use of such a lipophilic group will increase the chance of effective cell membrane and blood brain barrier permeability and in addition act as a selective targeting utility to reach mitochondria. TBTP-pantethine indeed showed increased membrane affinity[68-70]. However, such modification of pantethine did not increase the passive membrane permeability as compared to pantethine. Moreover, TBTP coupling did not increase the stability of pantethine, as pantetheinases present in fetal calf serum still degraded the TBTP-pantethine (Chapter 4; Figure 2). Although the identification of PANK-independent alternative CoA biosynthesis from pantethine changes the concept of vitamin-B5 being the only substrate to form cellular CoA, the instability of pantethine narrows its pharmacological benefits. It also clearly indicated the need to explore other possible sources for cellular CoA, which might be of therapeutic use to treat PKAN effectively.

CoA has been considered physiologically inert regarding functions outside the cell, and this molecule was never envisioned to be able to pass through the cell membrane, due to its bulky and highly electronegative nature. However, in an attempt to revert the protein acetylation defect induced by PKAN impairment in a *Drosophila in vitro* model,

direct supplementation of CoA in the external cell media revealed a complete rescue effect (Chapter 3; Figure 2)[49]. Moreover, CoA supplementation along with statins has been shown to have therapeutic benefit in treating hyperlipidemia[71,72]. These striking results made us

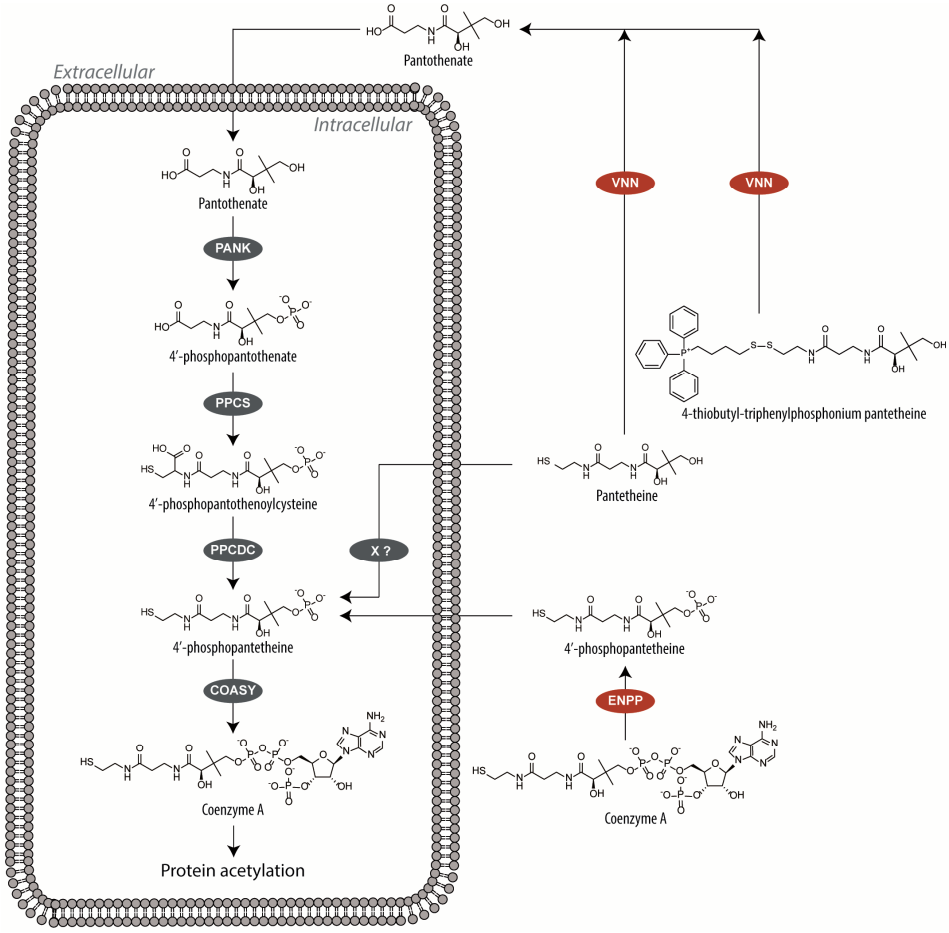

Figure 2: Over-view of research findings summarized in this thesis.
De novo CoA biosynthesis utilizing extracellular pantothenate and subsequently processed by pantothenate kinase (PANK), 4′-phosphopantothenoylcysteine synthetase (PPCS), (R)-4′-phospho-N-pantothenoylcysteine decarboxylase (PPCDC), and the bifunctional 4′-phosphopantetheine adenylyltransferase/dephospho-CoA kinase (COASY) to form CoA. Pantethine by-passes PANK and is processed by an unknown kinase/protein (X?) contributing to CoA biosynthesis (Chapter 2). Cellular CoA levels regulate the protein acetylation homeostasis inside the cell (Chapter 3). Pantetheinases or vanin (VNN) degrade both pantetheine and 4-thiobutyl-triphenylphosphonium pantetheine to pantothenate in the serum (Chapter 4). External supplemented CoA is converted into 4′-phosphopantethine by ecto-nucloetide-pyrophosphatases (ENPP) in the serum, 4′-phosphopantethine crosses the plasma membrane via passive diffusion and contributes to CoA biosynthesis in a COASY dependent manner (Chapter 5).

investigate whether such direct effects of CoA exist and if so what is the working mechanism behind it. To aid our study in a more precise way, we developed a sensitive chromatography method with selectivity for CoA and other related thiol molecules (Chapter 3 and 5).

The evidence presented in Chapter 5 of this thesis demonstrated that cells and organisms are indeed able to acquire CoA not only via the uptake of vitamin B5 but also via the direct supplementation of exogenous CoA. Our experimental data showed that CoA needs to be hydrolyzed extracellularly by exo-enzymes into 4'-phosphopantetheine (PPanSH), which is a biologically stable molecule and is taken up by cells. Intracellularly, PPanSH is converted back to CoA by the bifunctional enzyme COASY. This newly identified non-canonical source for CoA is independent of PANK and PPCDC. As described in Chapter 5, external supplementation of CoA, but not vitamin B5, rescued various phenotypes induced by impaired PANK and PPCDC in *Drosophila* model systems. The rescue effect of CoA in a validated *dPANK/fbl null* mutant and in a severely affected *dPPCDC Drosophila* mutant, but not *dCOASY* mutants, clearly revealed a potent working mechanism of CoA supplied via fly food. We also provided evidence that this mechanism is prevalent among diverse eukaryotic organisms and cells, including *C.elegans*, human cells and in mice. The proposed mechanism thus suggests a net flow of CoA via PPanSH between cells and between membrane-bound cellular compartments. Moreover, the rescue potency of CoA in reverting the female sterility in *dPPCDC* mutants clearly supports that the beneficial effect of PPanSH obtained from CoA is far reaching within an organism and is crossing various cellular barriers. In contrast to the instability of pantethine in human serum, PPanSH obtained via external CoA is found to be stable which indicates its promises to be an effective non-canonical substrate for CoA biosynthesis obtained via the circulation. The confirmation of endogenous PPanSH in mouse serum by both HPLC and mass spectrometry, substantiates the natural occurrence of this alternative source route in higher eukaryotes for CoA biosynthesis (Chapter 5). Exogenous CoA or PPanSH might not be beneficial for CoPAN therapy, as this alternative route requires active COASY. Nevertheless, CoA or PPanSH might provide a promising treatment possibility for PKAN.

Concluding remarks and perspectives

With the knowledge that CoA is not only a key molecule in metabolism playing a role in over 100 metabolic reactions, but that CoA also modulates the epigenome and at least activates one specific kinase (CaMKII)[9], the influence of this molecule in health and disease is most likely larger than ever anticipated. Further detailed investigation is yet required to understand whether impaired metabolism, abnormal protein acetylation or impaired activation of CaMKII or possible other proteins or a combinatorial effect causes the specific disease characteristics of PKAN and CoPAN. It is also important to understand the effects of mutations of *PANK2* and *COASY* with regards to their activity in both CoA biosynthesis but also in mitochondrial structural integrity. Since not all the mutations leading to PKAN correlates with loss of PANK2 activity[73,74] and with the use of CoPAN patient fibroblasts evidence was

presented that CoA is still being produced[31], mitochondrial dysfunction could be one of the leading cause of neurodegeneration. Hence, it is necessary for the future to perform further in-depth experiments to reveal precisely the link between the multifaceted influence of CoA and its related diseases, including PKAN and CoPAN.

It is also important to elucidate additional alternative possible ways to manipulate cellular CoA levels. *In vitro* studies have indicated that small molecules like palmitoyl carnitine and oleyl carnitine can activate PANK[75,76]. However, this has never been confirmed *in vivo*. Exploiting such small molecules as activators or repressors will be useful in our research to find possibilities to be able to manipulate CoA levels and this knowledge will be helpful to understand the role of CoA in cellular physiology and may lead to possible ways to treat PKAN and CoPAN. High throughput screening to identify regulators of the promoters of the genes coding for CoA biosynthesis enzymes could also be a useful approach[77]. Moreover, the use of pantethine or TBTP-pantetheine as novel alternative substrates for CoA production can be substantiated by combining them with pantetheinase inhibitors[78,79] to achieve clinical benefit.

Moreover, the use of HDAC inhibitors like TSA alone or in addition to pantethine or pantetheine derivatives might also provide efficient benefits compared to treatment with pantethine or pantetheine derivatives only. The new breakthrough in deciphering the working mechanism of externally supplemented CoA via PPanSH has broadened the scope of CoA research in both basic cell biology and in disease pathophysiology. Our experimental proof of PPanSH as a crucial molecule in reverting the PKAN phenotype may be a physiologically relevant process because we could detect endogenous PPanSH in fresh mouse serum. In clinical studies, CoA alone or with the addition of statins showed a beneficial effect in lowering plasma triglyceride levels in patients with moderate dyslipidemia and thereby acted as a natural hypolipidemic drug with no obvious adverse effect detected[71,72]. Our findings suggest that the above mentioned CoA mode of action in hyperlipidemia is via PPanSH. Recently, the *de novo* CoA biosynthesis pathway in microbes also obtained interest because of the fact that small molecules can be developed acting as novel antimicrobial drugs by specifically targeting the microbial CoA biosynthesis enzymes[80,81]. This is possible because prokaryotic CoA biosynthesis pathway enzymes vary in their sequences and structural integrity compared to the eukaryotic enzymes[80-82]. In addition, the development of CoA/PPanSH antimetabolities that can selectively inhibit the activity of parasite and microbial CoA biosynthesis enzymes and thereby reduce CoA levels and/or interfere with the species-specific CoA dependent processes will be promising to treat parasite and microbial infections[80,81]. Overall, this thesis summarized the diverse influence/function of CoA, apart from its most classically known metabolic role. It also reports about in-depth experimental outcomes to understand the role of impaired *de novo* CoA biosynthesis in cellular physiology. In addition, the finding of a possible alternative source for CoA biosynthesis like pantethine, PPanSH, and CoA provides a promising start to design a therapy for PKAN and other CoA related diseases.

REFERENCES

1. Wishart, D. S. et al. HMDB 3.0--The Human Metabolome Database in 2013. Nucleic acids research 41, D801-807, doi:10.1093/nar/gks1065 (2013).
2. DeBerardinis, R. J. & Thompson, C. B. Cellular metabolism and disease: what do metabolic outliers teach us? Cell 148, 1132-1144, doi:10.1016/j.cell.2012.02.032 (2012).
3. Naimi, M., Arous, C. & Van Obberghen, E. Energetic cell sensors: a key to metabolic homeostasis. Trends in endocrinology and metabolism: TEM 21, 75-82, doi:10.1016/j.tem.2009.09.003 (2010).
4. Donmez, G. & Outeiro, T. F. SIRT1 and SIRT2: emerging targets in neurodegeneration. EMBO molecular medicine 5, 344-352, doi:10.1002/emmm.201302451 (2013).
5. Mouchiroud, L., Houtkooper, R. H. & Auwerx, J. NAD(+) metabolism: a therapeutic target for age-related metabolic disease. Critical reviews in biochemistry and molecular biology 48, 397-408, doi:10.3109/10409238.2013.789479 (2013).
6. Lipmann, F., Kaplan, N. O. & Novelli, G. D. Chemistry and distribution of the coenzyme for acetylation (coenzyme A). Federation proceedings 6, 272 (1947).
7. Choudhary, C., Weinert, B. T., Nishida, Y., Verdin, E. & Mann, M. The growing landscape of lysine acetylation links metabolism and cell signalling. Nature reviews. Molecular cell biology 15, 536-550, doi:10.1038/nrm3841 (2014).
8. Wellen, K. E. et al. ATP-citrate lyase links cellular metabolism to histone acetylation. Science 324, 1076-1080, doi:10.1126/science.1164097 (2009).
9. McCoy, F. et al. Metabolic activation of CaMKII by coenzyme A. Molecular cell 52, 325-339, doi:10.1016/j.molcel.2013.08.043 (2013).
10. Leonardi, R., Zhang, Y. M., Rock, C. O. & Jackowski, S. Coenzyme A: back in action. Progress in lipid research 44, 125-153, doi:10.1016/j.plipres.2005.04.001 (2005).
11. Afshar, K., Gonczy, P., DiNardo, S. & Wasserman, S. A. fumble encodes a pantothenate kinase homolog required for proper mitosis and meiosis in *Drosophila* melanogaster. Genetics 157, 1267-1276 (2001).
12. Atomi, H., Tomita, H., Ishibashi, T., Yokooji, Y. & Imanaka, T. CoA biosynthesis in archaea. Biochemical Society transactions 41, 427-431, doi:10.1042/BST20120311 (2013).
13. Begley, T. P., Kinsland, C. & Strauss, E. The biosynthesis of coenzyme A in bacteria. Vitamins and hormones 61, 157-171 (2001).
14. Bosveld, F. et al. *De novo* CoA biosynthesis is required to maintain DNA integrity during development of the *Drosophila* nervous system. Human molecular genetics 17, 2058-2069, doi:10.1093/hmg/ddn105 (2008).
15. Daugherty, M. et al. Complete reconstitution of the human coenzyme A biosynthetic pathway via comparative genomics. The Journal of biological chemistry 277, 21431-21439, doi:10.1074/jbc.M201708200 (2002).
16. Kupke, T., Hernandez-Acosta, P. & Culianez-Macia, F. A. 4'-phosphopantetheine and coenzyme A biosynthesis in plants. The Journal of biological chemistry 278, 38229-38237, doi:10.1074/jbc.M306321200 (2003).
17. Zhou, B. et al. A novel pantothenate kinase gene (PANK2) is defective in Hallervorden-Spatz syndrome. Nature genetics 28, 345-349, doi:10.1038/ng572 (2001).
18. Rouault, T. A. Iron metabolism in the CNS: implications for neurodegenerative diseases. Nature reviews. Neuroscience 14, 551-564, doi:10.1038/nrn3453 (2013).
19. Prohaska, R. et al. Brain, blood, and iron: perspectives on the roles of erythrocytes and iron in neurodegeneration. Neurobiology of disease 46, 607-624, doi:10.1016/j.nbd.2012.03.006 (2012).
20. Schneider, S. A. & Bhatia, K. P. Syndromes of neurodegeneration with brain iron accumulation. Seminars in pediatric neurology 19, 57-66, doi:10.1016/j.spen.2012.03.005 (2012).
21. Thomas, M., Hayflick, S. J. & Jankovic, J. Clinical heterogeneity of neurodegeneration with brain iron accumulation (Hallervorden-Spatz syndrome) and pantothenate kinase-

associated neurodegeneration. Movement disorders : official journal of the Movement Disorder Society 19, 36-42, doi:10.1002/mds.10650 (2004).

22. Rubio, S., Whitehead, L., Larson, T. R., Graham, I. A. & Rodriguez, P. L. The coenzyme a biosynthetic enzyme phosphopantetheine adenylyltransferase plays a crucial role in plant growth, salt/osmotic stress resistance, and seed lipid storage. Plant physiology 148, 546-556, doi:10.1104/pp.108.124057 (2008).
23. Garcia, M., Leonardi, R., Zhang, Y. M., Rehg, J. E. & Jackowski, S. Germline deletion of pantothenate kinases 1 and 2 reveals the key roles for CoA in postnatal metabolism. PloS one 7, e40871, doi:10.1371/journal.pone.0040871 (2012).
24. Kuo, Y. M. et al. Deficiency of pantothenate kinase 2 (Pank2) in mice leads to retinal degeneration and azoospermia. Human molecular genetics 14, 49-57, doi:10.1093/hmg/ddi005 (2005).
25. Nakamura, T., Pluskal, T., Nakaseko, Y. & Yanagida, M. Impaired coenzyme A synthesis in fission yeast causes defective mitosis, quiescence-exit failure, histone hypoacetylation and fragile DNA. Open biology 2, 120117, doi:10.1098/rsob.120117 (2012).
26. Wu, Z., Li, C., Lv, S. & Zhou, B. Pantothenate kinase-associated neurodegeneration: insights from a *Drosophila* model. Human molecular genetics 18, 3659-3672, doi:10.1093/hmg/ddp314 (2009).
27. Faust, K. et al. Neuroprotective effects of compounds with antioxidant and anti-inflammatory properties in a *Drosophila* model of Parkinson's disease. BMC neuroscience 10, 109, doi:10.1186/1471-2202-10-109 (2009).
28. Lessing, D. & Bonini, N. M. Maintaining the brain: insight into human neurodegeneration from *Drosophila* melanogaster mutants. Nature reviews. Genetics 10, 359-370, doi:10.1038/nrg2563 (2009).
29. Pandey, U. B. & Nichols, C. D. Human disease models in *Drosophila* melanogaster and the role of the fly in therapeutic drug discovery. Pharmacological reviews 63, 411-436, doi:10.1124/pr.110.003293 (2011).
30. Rana, A. et al. Pantethine rescues a *Drosophila* model for pantothenate kinase-associated neurodegeneration. Proceedings of the National Academy of Sciences of the United States of America 107, 6988-6993, doi:10.1073/pnas.0912105107 (2010).
31. Dusi, S. et al. Exome sequence reveals mutations in CoA synthase as a cause of neurodegeneration with brain iron accumulation. American journal of human genetics 94, 11-22, doi:10.1016/j.ajhg.2013.11.008 (2014).
32. Jackowski, S. & Leonardi, R. Deregulated coenzyme A, loss of metabolic flexibility and diabetes. Biochemical Society transactions 42, 1118-1122, doi:10.1042/BST20140156 (2014).
33. Leonardi, R., Rock, C. O. & Jackowski, S. Pank1 deletion in leptin-deficient mice reduces hyperglycaemia and hyperinsulinaemia and modifies global metabolism without affecting insulin resistance. Diabetologia 57, 1466-1475, doi:10.1007/s00125-014-3245-5 (2014).
34. Leonardi, R., Rehg, J. E., Rock, C. O. & Jackowski, S. Pantothenate kinase 1 is required to support the metabolic transition from the fed to the fasted state. PloS one 5, e11107, doi:10.1371/journal.pone.0011107 (2010).
35. Zhang, Y. M. et al. Chemical knockout of pantothenate kinase reveals the metabolic and genetic program responsible for hepatic coenzyme A homeostasis. Chemistry & biology 14, 291-302, doi:10.1016/j.chembiol.2007.01.013 (2007).
36. Lin, M. T. & Beal, M. F. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 443, 787-795, doi:10.1038/nature05292 (2006).
37. Cai, H. et al. Metabolic dysfunction in Alzheimer's disease and related neurodegenerative disorders. Current Alzheimer research 9, 5-17 (2012).
38. Yin, F., Boveris, A. & Cadenas, E. Mitochondrial energy metabolism and redox signaling in brain aging and neurodegeneration.

Antioxidants & redox signaling 20, 353-371, doi:10.1089/ars.2012.4774 (2014).

39. Hortnagel, K., Prokisch, H. & Meitinger, T. An isoform of hPANK2, deficient in pantothenate kinase-associated neurodegeneration, localizes to mitochondria. Human molecular genetics 12, 321-327 (2003).
40. Johnson, M. A. et al. Mitochondrial localization of human PANK2 and hypotheses of secondary iron accumulation in pantothenate kinase-associated neurodegeneration. Annals of the New York Academy of Sciences 1012, 282-298 (2004).
41. Brunetti, D. et al. Pantothenate kinase-associated neurodegeneration: altered mitochondria membrane potential and defective respiration in Pank2 knock-out mouse model. Human molecular genetics 21, 5294-5305, doi:10.1093/hmg/dds380 (2012).
42. Choudhary, C. et al. Lysine acetylation targets protein complexes and co-regulates major cellular functions. Science 325, 834-840, doi:10.1126/science.1175371 (2009).
43. Yang, X. J. & Seto, E. HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention. Oncogene 26, 5310-5318, doi:10.1038/sj.onc.1210599 (2007).
44. Takahashi, H., McCaffery, J. M., Irizarry, R. A. & Boeke, J. D. Nucleocytosolic acetyl-coenzyme a synthetase is required for histone acetylation and global transcription. Molecular cell 23, 207-217, doi:10.1016/j.molcel.2006.05.040 (2006).
45. Zhang, M., Galdieri, L. & Vancura, A. The yeast AMPK homolog SNF1 regulates acetyl coenzyme A homeostasis and histone acetylation. Molecular and cellular biology 33, 4701-4717, doi:10.1128/MCB.00198-13 (2013).
46. Eisenberg, T. et al. Nucleocytosolic depletion of the energy metabolite acetyl-coenzyme a stimulates autophagy and prolongs lifespan. Cell metabolism 19, 431-444, doi:10.1016/j.cmet.2014.02.010 (2014).
47. Marino, G. et al. Regulation of autophagy by cytosolic acetyl-coenzyme A. Molecular cell 53, 710-725, doi:10.1016/j.molcel.2014.01.016 (2014).
48. Shi, L. & Tu, B. P. Acetyl-CoA induces transcription of the key G1 cyclin CLN3 to promote entry into the cell division cycle in Saccharomyces cerevisiae. Proceedings of the National Academy of Sciences of the United States of America 110, 7318-7323, doi:10.1073/pnas.1302490110 (2013).
49. Siudeja, K. et al. Impaired Coenzyme A metabolism affects histone and tubulin acetylation in *Drosophila* and human cell models of pantothenate kinase associated neurodegeneration. EMBO molecular medicine 3, 755-766, doi:10.1002/emmm.201100180 (2011).
50. Graff, J. & Tsai, L. H. Histone acetylation: molecular mnemonics on the chromatin. Nature reviews. Neuroscience 14, 97-111, doi:10.1038/nrn3427 (2013).
51. Garbes, L., Riessland, M. & Wirth, B. Histone acetylation as a potential therapeutic target in motor neuron degenerative diseases. Current pharmaceutical design 19, 5093-5104 (2013).
52. Suzuki, K. & Koike, T. Mammalian Sir2-related protein (SIRT) 2-mediated modulation of resistance to axonal degeneration in slow Wallerian degeneration mice: a crucial role of tubulin deacetylation. Neuroscience 147, 599-612, doi:10.1016/j.neuroscience.2007.04.059 (2007).
53. Govindarajan, N. et al. Reducing HDAC6 ameliorates cognitive deficits in a mouse model for Alzheimer's disease. EMBO molecular medicine 5, 52-63, doi:10.1002/emmm.201201923 (2013).
54. Chen, C. C. et al. Acetylated lysine 56 on histone H3 drives chromatin assembly after repair and signals for the completion of repair. Cell 134, 231-243, doi:10.1016/j.cell.2008.06.035 (2008).
55. Das, C., Lucia, M. S., Hansen, K. C. & Tyler, J. K. CBP/p300-mediated acetylation of histone H3 on lysine 56. Nature 459, 113-117, doi:10.1038/nature07861 (2009).
56. Saha, R. N. & Pahan, K. HATs and HDACs in neurodegeneration: a tale of disconcerted acetylation homeostasis. Cell death and differentiation 13, 539-550, doi:10.1038/sj.cdd.4401769 (2006).

57. Vempati, R. K. et al. p300-mediated acetylation of histone H3 lysine 56 functions in DNA damage response in mammals. The Journal of biological chemistry 285, 28553-28564, doi:10.1074/jbc.M110.149393 (2010).

58. Yuan, J., Pu, M., Zhang, Z. & Lou, Z. Histone H3-K56 acetylation is important for genomic stability in mammals. Cell Cycle 8, 1747-1753 (2009).

59. Jeppesen, D. K., Bohr, V. A. & Stevnsner, T. DNA repair deficiency in neurodegeneration. Progress in neurobiology 94, 166-200, doi:10.1016/j.pneurobio.2011.04.013 (2011).

60. Katyal, S. & McKinnon, P. J. DNA repair deficiency and neurodegeneration. Cell Cycle 6, 2360-2365 (2007).

61. Reynolds, J. J. & Stewart, G. S. A nervous predisposition to unrepaired DNA double strand breaks. DNA repair 12, 588-599, doi:10.1016/j.dnarep.2013.04.011 (2013).

62. Sun, L. et al. Effects of cysteamine on MPTP-induced dopaminergic neurodegeneration in mice. Brain research 1335, 74-82, doi:10.1016/j.brainres.2010.03.079 (2010).

63. Cornille, E. et al. Enhancement of L-3-hydroxybutyryl-CoA dehydrogenase activity and circulating ketone body levels by pantethine. Relevance to dopaminergic injury. BMC neuroscience 11, 51, doi:10.1186/1471-2202-11-51 (2010).

64. Kaskow, B. J., Proffitt, J. M., Blangero, J., Moses, E. K. & Abraham, L. J. Diverse biological activities of the vascular non-inflammatory molecules - the Vanin pantetheinases. Biochemical and biophysical research communications 417, 653-658, doi:10.1016/j.bbrc.2011.11.099 (2012).

65. Dupre, S., Graziani, M. T., Rosei, M. A., Fabi, A. & Del Grosso, E. The enzymatic breakdown of pantethine to pantothenic acid and cystamine. European journal of biochemistry / FEBS 16, 571-578 (1970).

66. Wittwer, C. T. et al. Purification and properties of a pantetheine-hydrolyzing enzyme from pig kidney. The Journal of biological chemistry 258, 9733-9738 (1983).

67. Brunetti, D. et al. Pantethine treatment is effective in recovering the disease phenotype induced by ketogenic diet in a pantothenate kinase-associated neurodegeneration mouse model. Brain : a journal of neurology 137, 57-68, doi:10.1093/brain/awt325 (2014).

68. Burns, R. J., Smith, R. A. & Murphy, M. P. Synthesis and characterization of thiobutyltriphenylphosphonium bromide, a novel thiol reagent targeted to the mitochondrial matrix. Archives of biochemistry and biophysics 322, 60-68, doi:10.1006/abbi.1995.1436 (1995).

69. Gane, E. J. et al. The mitochondria-targeted anti-oxidant mitoquinone decreases liver damage in a phase II study of hepatitis C patients. Liver international : official journal of the International Association for the Study of the Liver 30, 1019-1026, doi:10.1111/j.1478-3231.2010.02250.x (2010).

70. Mehiri, M. et al. A "ready-to-use" fluorescent-labelled-cysteine-TBTP (4-thiobutyltriphenylphosphonium) synthon to investigate the delivery of non-permeable PNA (peptide nucleic acids)-based compounds to cells. Bioorganic chemistry 35, 313-326, doi:10.1016/j.bioorg.2007.01.002 (2007).

71. Chen, Y. Q., Zhao, S. P., Chen, J. Z. & Lai, J. The effects of coenzyme A on serum lipids in patients with hyperlipidemia: results of a multicenter clinical trial. The Journal of clinical endocrinology and metabolism 98, E275-278, doi:10.1210/jc.2012-2702 (2013).

72. Lai, J., Wu, B., Xuan, T., Liu, Z. & Chen, J. Efficacy and tolerability of adding coenzyme A 400 U/d capsule to stable statin therapy for the treatment of patients with mixed dyslipidemia: an 8-week, multicenter, double-blind, randomized, placebo-controlled study. Lipids in health and disease 13, 1, doi:10.1186/1476-511X-13-1 (2014).

73. Kotzbauer, P. T., Truax, A. C., Trojanowski, J. Q. & Lee, V. M. Altered neuronal mitochondrial coenzyme A synthesis in neurodegeneration with brain iron accumulation caused by abnormal processing, stability, and catalytic activity of mutant pantothenate kinase 2. The Journal of neuroscience : the official journal of the Society for Neuroscience 25, 689-698, doi:10.1523/JNEUROSCI.4265-04.2005 (2005).

74. Zhang, Y. M., Rock, C. O. & Jackowski, S. Biochemical properties of human pantothenate kinase 2 isoforms and mutations linked to pantothenate kinase-associated neurodegeneration. The Journal of biological chemistry 281, 107-114, doi:10.1074/jbc.M508825200 (2006).
75. Leonardi, R., Rock, C. O., Jackowski, S. & Zhang, Y. M. Activation of human mitochondrial pantothenate kinase 2 by palmitoylcarnitine. Proceedings of the National Academy of Sciences of the United States of America 104, 1494-1499, doi:10.1073/pnas.0607621104 (2007).
76. Leonardi, R. et al. Modulation of pantothenate kinase 3 activity by small molecules that interact with the substrate/allosteric regulatory domain. Chemistry & biology 17, 892-902, doi:10.1016/j.chembiol.2010.06.006 (2010).
77. Hughes, J. P., Rees, S., Kalindjian, S. B. & Philpott, K. L. Principles of early drug discovery. British journal of pharmacology 162, 1239-1249, doi:10.1111/j.1476-5381.2010.01127.x (2011).
78. Jansen, P. A. et al. Discovery of small molecule vanin inhibitors: new tools to study metabolism and disease. ACS chemical biology 8, 530-534, doi:10.1021/cb3006424 (2013).
79. Schalkwijk, J. & Jansen, P. Chemical biology tools to study pantetheinases of the vanin family. Biochemical Society transactions 42, 1052-1055, doi:10.1042/BST20140074 (2014).
80. Moolman, W. J., de Villiers, M. & Strauss, E. Recent advances in targeting coenzyme A biosynthesis and utilization for antimicrobial drug development. Biochemical Society transactions 42, 1080-1086, doi:10.1042/BST20140131 (2014).
81. Pett, H. E. et al. Novel pantothenate derivatives for anti-malarial chemotherapy. Malaria journal 14, 169, doi:10.1186/s12936-015-0673-8 (2015).
82. Genschel, U. Coenzyme A biosynthesis: reconstruction of the pathway in archaea and an evolutionary scenario based on comparative genomics. Molecular biology and evolution 21, 1242-1251, doi:10.1093/molbev/msh119 (2004).