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Discovery and functional characterisation of protein CoAlation and the antioxidant function of coenzyme A

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ABSTRACT

Coenzyme A (CoA) is an essential cofactor in all living cells which plays critical role in cellular metabolism, the regulation of gene expression and the biosynthesis of major cellular constituents. Recently, CoA was found to function as a major antioxidant in both prokaryotic and eukaryotic cells. This unconventional function of CoA is mediated by a novel post-translational modification, termed protein CoAlation. This review will highlight the history of this discovery, current knowledge, and future directions on studying molecular mechanisms of protein CoAlation and whether the antioxidant function of CoA is associated with pathologies, such as neurodegeneration and cancer.

Introduction

Coenzyme A (CoA) is a universal metabolic cofactor indispensable for the viability of all living cells [1]. It was discovered by Fritz Lipmann in the middle of last century and this seminal discovery earned him the Nobel Prize which he co-shared with Hans Krebs who deciphered the citric acid cycle. CoA is produced in prokaryotic and eukaryotic cells by a conservative pathway of five enzymatic reactions, involving conjugation of ATP, pantothenate (Vitamin B5) and cysteine (Fig. 1A). The presence of the ADP moiety and the thiol group at a flexible pantetheine tail allows CoA to be involved in diverse biochemical reactions and the biosynthesis of metabolically active CoA thioesters, including acetyl-CoA, malonyl-CoA, HMG-CoA among others [1–3]. Numerous anabolic and catabolic pathways have been found to involve CoA and CoA thioesters. These include the biosynthesis of fatty acids, ketone bodies and cholesterol (malonyl-CoA and HMG-CoA), amino acid metabolism (propionyl-CoA and succinyl-CoA), fatty acid oxidation (acyl-CoA and acetyl-CoA), biosynthesis of ATP and neurotransmitter acetylcholine (acetyl-CoA) etc. (Fig. 1B).

A novel function of CoA in redox regulation has been recently discovered in eukaryotic and prokaryotic cells (Fig. 1B) [4–7]. Under oxidative or metabolic stress, CoA was found to use the thiol group for covalent modification of oxidized cysteine residues, protecting them from irreversible overoxidation and facilitating the antioxidant response. This modification was termed protein CoAlation and shown to be widespread and reversible. To date, over 2000 proteins have been found to be CoAlated in bacterial and mammalian cells/tissues exposed to oxidative or metabolic stress. Protein CoAlation is known to modulate the conformation, activity, subcellular localization of modified proteins, and to protect them from irreversible overoxidation and subsequent degradation [5,7].

In mammalian cells and tissues, the level of CoA/CoA derivatives is not at steady-state and changes are mediated via the level of transcription and translation of genes encoding biosynthetic enzymes, regulation of their activities by signaling pathways, feedback mechanisms, degradation and interconversion of CoA and CoA thioesters [8–10]. The highest CoA levels are found in the liver, heart and brown adipose tissue, followed by kidney and brain. In these organs and tissues, CoA and acetyl-CoA are the largest components of the total CoA pool. Acetyl-CoA occupies a strategic position in ATP production, biosynthesis of lipids, cholesterol and acetylcholine, as well as the regulation of gene expression via protein acetylation (Fig. 1B). Acetyl-CoA is produced by

Abbreviations: CoA, coenzyme A; COASY/CoAsy, CoA synthase; DPCK, dephospho-CoA kinase; EDC4, enhancer of mRNA-decapping protein 4; GSH, glutathione; LMW, low molecular weight; NBIA, neurodegeneration with brain iron accumulation; mTOR, mammalian target of rapamycin; NADH, nicotinamide adenine dinucleotide; PANK, pantothenate kinase; PI3K, phosphoinositide 3-kinase; PPAT, 4′-phosphopantetheine adenylyltransferase; PPCDC, 4′-phosphopantothenoylcysteine decarboxylase; PPCS, 4′-phosphopantothenoylcysteine synthase; ROS, reactive oxygen species; Shp2PTP, Src homology 2 domain-containing protein tyrosine phosphatase; S6K, ribosomal S6 kinase.

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different mechanisms in mitochondria and cytosol (Fig. 1C) [11]. In mitochondria, the pathway of acetyl-CoA biosynthesis involves the oxidation of pyruvate by the mitochondrial pyruvate dehydrogenase complex, accompanied by the reduction of NAD^+. The production of cytosolic acetyl-CoA is mediated by acetyl-CoA synthases, which ligate acetate and CoA through the consumption of ATP.

The distribution of CoA in different compartments of mammalian cells differs significantly and reflects the variety of processes in which it is implicated. The highest concentration of CoA is found in mitochondria (2–5 mM) and peroxisomes (0.7 mM), whereas significantly lower levels of CoA are detected in the cytosol and nucleus (0.05 - 0.14 mM) [1].

Various human pathologies, including diabetes, Reye's syndrome, cancer, vitamin B12 deficiency and cardiac hypertrophy have been associated with abnormal biosynthesis and homeostasis of CoA/CoA derivatives [12–14]. Inborn mutations in pantothenate kinase 2 (PANK2) and CoA synthase (COASY) result in the development of a severe neurodegenerative disease called neurodegeneration with brain iron accumulation (NBIA), suggesting that dysregulation of CoA biosynthesis plays a crucial role in maintaining neuronal function and homeostasis in the central nervous system [15–16].

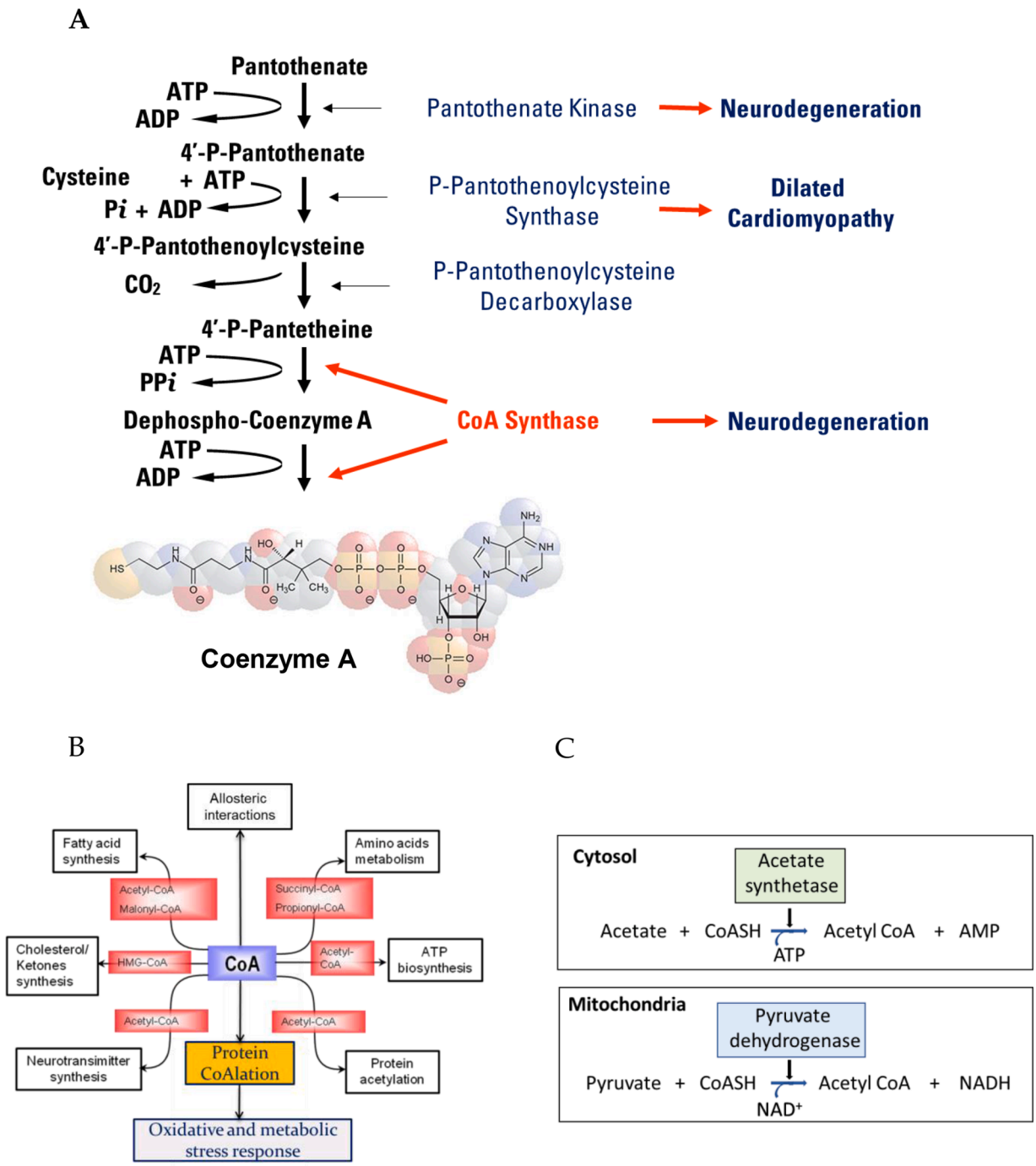

Fig. 1. A) Schematic diagram of the CoA biosynthetic pathway in mammalian cells. CoA synthase (in red) is a bifunctional enzyme which mediates the last two steps of CoA biosynthesis. In-born mutations in PANK2 and COASY lead to neurodegeneration with brain iron accumulation, and in PPCS – to dilated cardiomyopathy. **B)** Cellular functions of CoA and CoA thioesters in the regulation of metabolism, gene expression and the antioxidant response. **C)** The CoA thioester, acetyl-CoA, is produced by different mechanisms in mitochondria and cytosol.

Molecular cloning and characterization of CoA synthase

We and two other laboratories simultaneously reported in the beginning of this century the identification of mammalian CoA synthase (CoAsy), using molecular and bioinformatics approaches [17–19]. The coding sequence of CoAsy was found to possess phosphopantetheine adenylyltransferase (PPAT) and dephospho-CoA kinase (dPCoAK) domains which mediate the last two steps of CoA biosynthesis. These original studies completed the task of defining molecular identity of genes and enzymes involved in the CoA biosynthetic pathway in mammalian cells (Fig. 1A). They have also provided research tools for investigating subcellular localization and regulation of CoAsy in cellular response to extracellular stimuli and stress and exploring its role in the formation of the CoA biosynthetic complex. Building on these advances, we demonstrated that mCoAsy is localized to mitochondria and the N-terminal stretch of hydrophobic amino acids is responsible for targeting the enzyme to outer membrane of mitochondria (OMM) in the way that both enzymatic domains face the cytosol [20]. In addition to be found on the OMM, CoASy was also shown to be localized in the cytosol and in the mitochondrial matrix [21,22].

Furthermore, both enzymatic activities of CoAsy were shown to be potently activated by phosphatidylcholine and phosphatidylethanolamine, which are the main components of the OMM. Subsequent studies revealed that mCoAsy associates with and is regulated by several signaling proteins, including ribosomal S6 kinase (S6K), class 1A phosphatidylinositol 3-kinase (PI3K), Src family kinases and Shp2 tyrosine phosphatase (Shp2PTP) [23–25]. The enhancer of mRNA-decapping protein 4 (EDC4), which is a central scaffold component of processing bodies in stress response, was found to be in complex with mCoAsy and to regulate its dpCoAK activity [26]. These findings suggest that, in addition to pantothenate kinase (PanK), which is known as a master regulator of CoA biosynthesis, mCoAsy has a potential to function as a rate-limiting enzyme in the CoA biosynthetic pathway.

Since the first three enzymes of the CoA biosynthetic pathway, except for PanK2, are localized in the cytosolic/nuclear compartments, we proposed that CoAsy can function as a scaffold on the OMM for the formation of the CoA biosynthetic complex [27]. Using the *in situ* proximity ligation assay, we showed that the enzymes of the CoA biosynthetic pathway are located in close proximity in exponentially growing HEK293/Pank1β cells and oxidative stress promotes their association [28]. Furthermore, the association of endogenous enzymes of the CoA biosynthetic pathway in A549 lung cancer cells was shown to be strongly induced in response to oxidative stress and serum starvation, whereas treatment of serum-starved cells with insulin and fibroblast growth factor 2 (FGF2) downregulated their assembly [28]. These findings are in line with the reported formation of the CoA-synthesizing protein complex (CoA-SPC) in yeast [29] and suggest that clustering of the CoA biosynthetic enzymes around CoAsy at the OMM may facilitate efficient catalysis of CoA biosynthesis via channeling of substrates and intermediates. The production of CoA at the OMM makes it available in the cytosol and for subsequent diffusion into the nucleus. It can also provide high local concentration of CoA at OMM to facilitate its import into mitochondria via the SLC25A42 transporter in exchange for (deoxy) adenine nucleotides and adenosine 3′,5′-diphosphate [30].

Discovery of protein CoAlation and the antioxidant function of CoA

Cellular functions of CoA are determined by the presence of a nucleotide moiety and a reactive thiol group at the tip of pantetheine tail. Since the discovery of CoA, the research has been mainly focused on investigating the role of its thiol group in the interaction with a diverse range of carboxylic acids, resulting in the formation of high-energy CoA thioesters [1,2]. In contrast, the function of CoA as a low molecular weight (LMW) thiol in the antioxidant defense has been under-investigated, when compared to glutathione (GSH) which is the most abundant and most studied LMW thiol. In Gram-positive bacteria, bacillithiol and mycothiol are produced instead of GSH, while CoA is a major thiol [31]. The presence of a thiol group and the induction of CoA biosynthesis by oxidizing agents and serum starvation prompted us to investigate whether, in addition to be a key metabolic cofactor, CoA is also involved in redox regulation. The discovery and the study of the antioxidant function of CoA was hampered by the absence of specific reagents and methodologies. The lack of anti-CoA antibodies on the market and in research laboratories across the world was the main bottleneck for advancing research on this topic. By applying several immunization strategies and hybridoma screens, we identified two CoA-specific hybridoma clones [32]. Generated monoclonal antibodies from both clones recognized in the ELISA assay CoA and CoA thioesters, but not ADP, pantetheine or dephospho-CoA. They were also specific in recognizing CoA bound to BSA via disulfide bond in Western blot analysis under non-reducing conditions. One of these hybridoma clones (1F10) has been particularly useful in detecting CoA bound to proteins in other immunological assays, including immunoprecipitation, immunofluorescence, and immunohistochemistry [32]. The availability of highly specific anti-CoA 1F10 mAb allowed us to demonstrate for the first time that CoA employs its thiol group for covalent modification of oxidized cysteine residues *in vitro* and in cells under oxidative stress [4]. We termed this novel post-translational modification protein CoAlation. Initial studies revealed significant induction of protein CoAlation in rat primary cardiomyocytes exposed to several oxidizing agents. These novel findings were further validated in HEK293/Pank1β cells under oxidative stress and in isolated rat hearts perfused with hydrogen peroxide [4]. The use of HEK293/Pank1β cells in studying protein CoAlation was dictated by our unexpected findings that the level of total CoA in established cell lines is significantly lower when compared to primary cells or rat tissues [4,33]. Therefore, we generated HEK293 cells with stable overexpression of Pank1β, the main rate-limiting enzyme in promoting CoA biosynthesis. The level of CoA in HEK293/Pank1β cells was comparable to that in primary cells and in rat tissues. This cell model has been used for studying protein CoAlation under various experimental conditions, including metabolic stress. We showed that culturing HEK293/Pank1β cells in media with low glucose (5 mM) and without pyruvate results in significant induction of protein CoAlation. These findings led us to examine the pattern of protein CoAlation in animal models under physiological and pathological metabolic conditions. The increase in covalent protein modification by CoA was found in the rat liver after fasting for 24 h. We also showed that feeding high fat/high sucrose diet for 1 week resulted in a substantial decrease in protein CoAlation in rat liver. In agreement with these findings, protein CoAlation was also significantly decreased in the liver of genetically obese ob/ob mice when compared with wild-type controls [4].

It was rewarding to observe that protein CoAlation is a widespread post-translational modification (PTM) which occurs not only in mammalian cells and tissues, but also in other model organisms, including bacteria, yeast, amoeba, worms and flies exposed to oxidative or metabolic stress [34–36]. In line with other PTMs, such as phosphorylation, acetylation or glutathionylation, protein CoAlation was also found to be a reversible regulatory event [4,6]. We showed that diamide or H_2O_2-induced protein CoAlation is reversed to the background level within 5 min after removing oxidizing agents from cultured cells [4]. In addition, the pretreatment of cells with known antioxidants, such as N-acetyl-L-cysteine (NAC) or vitamin C, results in the inhibition of ROS-induced protein CoAlation [4].

The second bottleneck in advancing research on the antioxidant function of CoA was the lack of methodologies for the identification of CoAlated proteins. Generation of anti-CoA mAb capable of immunoprecipitating CoAlated tryptic peptides was critical for the development of mass spectrometry-based strategy for revealing the identity of CoA-modified proteins. This was not a straightforward task, as the LC–MS/MS analysis of CoAlated peptides immunoprecipitated with anti-CoA mAb revealed only a few matching fragment ion spectra with an

increase in 765 Da, corresponding to covalently bound CoA to a cysteine residue. The issue was caused by the fragmentation of CoA in CoAlated peptides during LC–MS/MS, making their identification (Cys + 765) by the MaxQuant software unfeasible [4]. To overcome this problem, immunoprecipitated CoAlated peptides were digested with Nudix7 (CoA/acyl CoA diphosphatase) to remove the ADP moiety [37]. The LC–MS/MS analysis of CoAlated peptides digested with Nudix7 results in a distinctive MS/MS fragmentation signature of Cys + 356, corresponding to covalently attached 4PP. The complexity of the developed protocol highlighted why CoAlated peptides have not been identified in numerous proteome-wide screens, employed to search for PTMs.

Functional consequences of protein CoAlation

To date, nearly 2100 CoAlated proteins have been identified using the developed methodology in bacteria and mammalian cells/tissues exposed to oxidative or metabolic stress. Bioinformatics analysis of CoAlated proteins showed that they are predominantly involved in metabolic processes (over 65%), as well as stress response and protein synthesis [5,7]. These novel findings raised an important question: what is functional relevance of protein CoAlation in health and pathologies associated with oxidative stress? The identification of *in vivo* CoAlated proteins and the development of an efficient *in vitro* CoAlation assay provided the basis for studying the effect of covalently bound CoA on the function of modified proteins (Fig. 2). Enzymatic activities of several metabolic and signaling proteins, which possess cysteine residue in the catalytic pocket, were shown to be inhibited by *in vitro* CoAlation [4]. These include aconitase, creatine kinase (CK), pyruvate dehydrogenase kinase 2 (PDK2), glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and hydroxymethylglutaryl-CoA synthase (HMGCS2), Aurora A kinase and metastasis suppressor protein NME1 [4,38-40]. An allosteric mode of activation was observed for some protein kinases which are CoAlated under oxidative stress at cysteine residues located outside the catalytic pocket (unpublished studies). Many transcription factors and transcriptional regulators, especially in bacteria, were shown to be CoAlated in cellular response to oxidative stress. *In vitro* CoAlation of a redox-sensing transcription factor AgrA at cysteine residue locate in the DNA-binding domain, revealed the inhibition of its binging to DNA, which is most likely mediated by steric interference [40].

It is well-established that prolong oxidative stress may cause over-oxidation of surface cysteine residues to sulphonic acids, leading to the inactivation and subsequent degradation of modified proteins. We showed that *in vitro* CoAlation of recombinant *S. aureus* GAPDH prevents irreversible overoxidation of catalytic cysteine residue by hydrogen peroxide [6]. The inhibition of GAPDH activity by CoAlation was fully reverted in the presence of DTT, suggesting a reversible mode of recovery from oxidative stress mediated by deCoAlation enzyme(s).

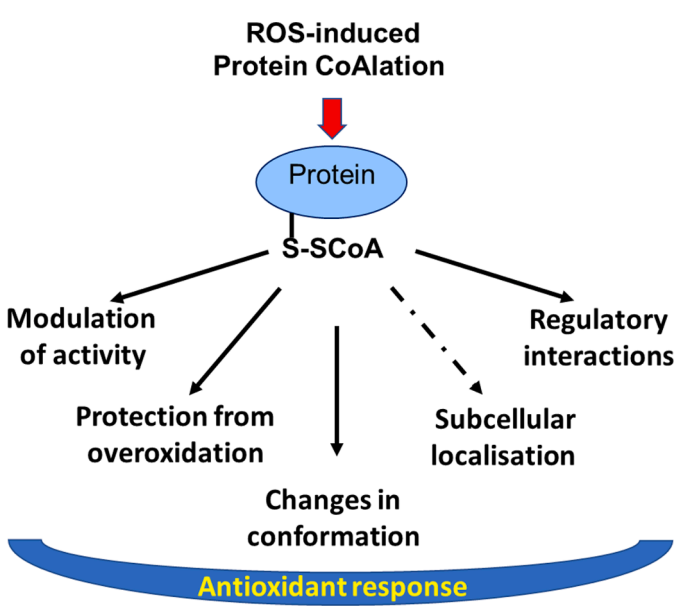

Fig. 2. Emerging functions of protein CoAlation. Oxidative stress promotes protein CoAlation and the antioxidant response mediated by CoA.

Bioinformatic analysis of CoAlated proteins revealed that modified cysteine residues are often located in close vicinity to motifs which determine their subcellular localization, including nuclear localization signal. Mutational analysis of one of these cysteine residues in a protein kinase involved in the cell cycle regulation was shown to modulate its nuclear localization in cellular response to oxidizing agents (unpublished data).

Covalent PTMs are known to induce significant conformational changes of modified proteins, and it is the same for protein CoAlation. Biochemical, biophysical, crystallographic and cell biology approaches were employed to reveal a unique mode of binding and regulation of Aurora A kinase by CoA, involving significant conformational changes [38]. In this study, CoA was shown to bind to Aurora A kinase under oxidative stress via specific ADP moiety interaction with the ATP binding pocket, and covalent modification of Cys290 in the activation loop by the thiol group of the flexible pantetheine tail.

Covalent protein modifications by phosphate, acetate or methyl groups create specific binding sites for regulatory protein interactions and the formation of multienzyme complexes, mediated by Src homology 2 (SH2), bromodomain and chromodomain respectively. Covalent binding of bulky CoA to oxidized cysteine residues may expose the ADP moiety on the surface of modified proteins, creating a novel site for regulatory interactions. It is plausible to speculate that Rossmann-fold containing proteins can recognize CoAlated proteins via specific recognition of the ADP moiety, and therefore promoting the formation of the antioxidant response signaling complex.

Elucidation of the CoAlation/deCoAlation cycle

The discovery of protein CoAlation and the antioxidant function of CoA raises a fundamentally important question: what are the molecular mechanisms of protein CoAlation/deCoAlation? By analogy to protein S-Glutahionylation which has been extensively studied in the last three decades, the following enzymes can be involved in the regulation of the protein CoAlation cycle: a) CoA transferase(s), promoting protein CoAlation; b) CoAredoxin(s), facilitating protein deCoAlation; c) CoA dependent peroxidase(s) – to reduce H2O2 and peroxide radicals; and d) CoA disulfide reductase(s) – regenerating CoASH from CoA disulfide dimer (Fig. 3).

The forward reaction of protein CoAlation and CoA transferases

The pKa value for the CoA thiol group is high (9.83), which is determined by its position at the tip of the pantetheine tail [41]. Therefore, CoA exists predominantly in its unreactive thiol form at physiological pH, which protects it from auto-oxidation and oxidation to the sulfenic acid state. To be engaged in the nucleophilic attack, the CoA thiol group has to be activated to a thiolate state by enzyme(s) which can reduce the pKa value of the CoA thiol and facilitate covalent modification of cellular targets. The proposed mechanism was reported for GSH in complex with glutathione S-transferase-pi [42]. The identity of CoA transferase(s) remains to be revealed and their involvement in redox regulation investigated.

Furthermore, CoA has a good capability to buffer oxidative stress in cells as its redox potential (−234 mV) is near to that of glutathione (−240 mV) which is the best-studied LMW thiol in the antioxidant response [43,44].

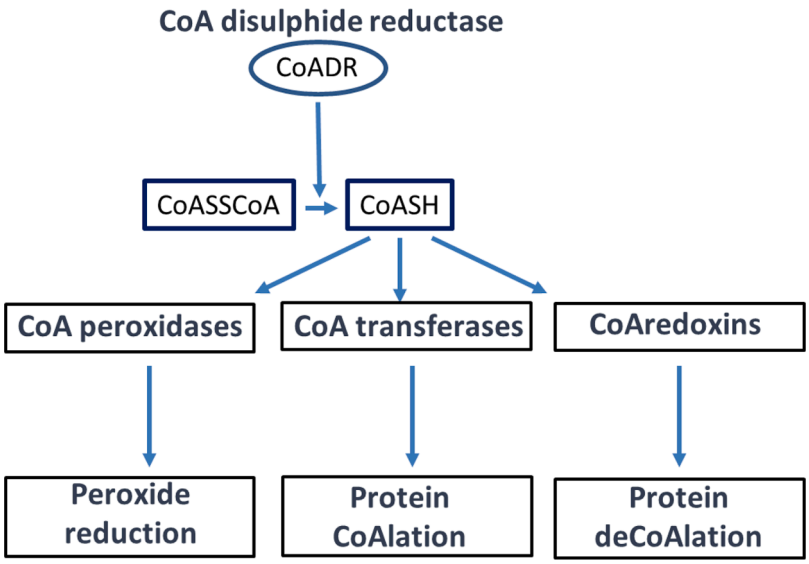

Fig. 3. Molecular dissection of the CoAlation/deCoAlation cycle. By analogy to protein S-glutathionylation, the antioxidant function of CoA may involve following enzymes: a) CoA transferase(s); b) CoAredoxin(s); c) CoA-dependent peroxidase(s); and d) CoA disulfide reductase(s).

Defining the mechanism of protein deCoAlation

Protein CoAlation was shown to be a reversible PTM in both prokaryotes and eukaryotes. In mammalian cells, extensive protein CoAlation induced by H_2O_2 or diamide is rapidly reversed to basal levels upon removal of the oxidants [4]. The same phenomenon was observed in bacteria, where the induction of protein CoAlation by glucose deprivation was quickly reversed with the re-addition of glucose to starved bacterial cultures [6]. The development of an *in vitro* assay for measuring deCoAlation activity in cell and tissue extracts allowed us to demonstrate that the removal of covalently bound CoA from proteins is enzymatically mediated in a similar way as protein deglutathionylation is facilitated by glutaredoxins [45]. Therefore, we termed proteins possessing the deCoAlation activity CoAredoxins(s) and their purification from bacteria and mammalian cells and tissues is currently in progress. We anticipate that CoAredoxins would possess a nucleotide and/or pantetheine binding folds allowing specific recognition of protein-bound CoA, and a thioredoxin fold which can catalyze the removal of CoA from CoAlated proteins.

CoA-dependent peroxidase(s) – reduce H_2O_2 and peroxide radicals

The antioxidant response in cells involves several mechanisms: a) direct scavenging of reactive oxygen species: b) inhibition of enzymes that produce ROS, or chelation of metals which promote free radical formation; and c) enhancing of antioxidant defenses. A diverse range of enzymes, regulatory proteins, cofactors and LMW thiols are involved in maintaining redox homeostasis, including superoxide dismutase, catalase, peroxiredoxin, thioredoxin and glutaredoxin systems, LMW thiols etc. The family of non-heme peroxidases comprises of two major subfamilies: peroxiredoxins (PRDXs) and glutathione-dependent peroxidases (GPxs). In human, eight glutathione peroxidases (GPx1–8) have been identified and shown to reduce hydrogen peroxide to water and lipid hydroperoxides to their corresponding alcohols [46]. The discovery of the antioxidant function of CoA implies the existence of enzymes which can reduce hydrogen peroxide and lipid hydroperoxides in a CoA-dependent manner. Indeed, we have recently identified an enzyme with CoA-dependent peroxidase activity and termed it CoAPx (unpublished data).

CoA disulfide reductase – regenerating CoASH from CoA disulfide dimer

The identification of eukaryotic CoADR is long-overdue. Purification and molecular cloning of *S. aureus* CoADR was reported more than 20 years ago [47]. Since then, CoADRs were identified in a diverse range of prokaryotes and archaea, but not in eukaryotes. Molecular cloning and crystallographic studies of prokaryotic/archaeal CoADRs showed that they belong to the family of pyridine nucleotide-disulfide oxidoreductases, which catalyze the conversion of CoA disulfide dimer (CoASSCoA, CoAD) into two molecules of CoASH, using the reducing power of NADH or NADPH [48]. This function requires the presence of the NADH/-NADPH and FAD binding domains and a catalytic cysteine residue which forms a Cys-S-S-CoA intermediate reduced to CoASH by NADH/NADPH via the FAD cofactor. Bioinformatics searches of mammalian databases with bacterial CoADR sequences have not led to the identification CoADR orthologs in eukaryotes. Therefore, the conventional purification of CoADR activity from mammalian cells/tissues, followed by mass spectrometry is required for the identification and subsequent characterization of mammalian CoADR.

Published studies describing the level of CoAD and the CoA:CoAD ratio in eukaryotic cells/tissues are very limited. It remains to be demonstrated that the CoA:CoAD ratio changes in cells/tissues exposed to oxidative or metabolic stress when compared to controls. The development of appropriate HPLC and/or mass spectrometry methodologies will be required to achieve this task, while mutational and knockout studies of identified mammalian CoADR will validate these findings.

Conclusions and future perspectives

We hypothesize that CoA functions as a key metabolic integrator under physiological conditions, but switches to be a major cellular antioxidant in response to oxidative or metabolic stress. Understanding the molecular mechanisms by which CoA mediates the antioxidant response in prokaryotic and eukaryotic cells is a major challenge of this emerging field of research. This will require the identification of enzymes involved in the CoAlation/deCoAlation cycle in prokaryotes and eukaryotes. The characterization of identified enzymes is expected to be facilitated by current advances in genome sequencing and editing and the availability of research tools, cell-based and animal models as well as opportunities for collaborative interactions.

At present, covalent modification of protein-reactive thiols with CoA

is known to include two mechanisms: a) the thiol-disulfide exchange reaction, which relies on the cellular level of CoASSCoA and shifting the CoASH:CoASSCoA redox balance toward a more oxidizing state; b) the conversion of protein thiolates to sulfenic acids, which involves the two-electron oxidation of a cysteine thiolate under oxidative stress. It remains to investigate whether other molecular mechanisms are implicated in mediating protein CoAlation *in vivo* via the formation of reactive thiol derivates, such as sulfenyl-amides, thiyl radicals, S-nitrosylated thiols, and thiosulfinates.

Many proteins implicated in the regulation of cell growth, proliferation and survival have been found CoAlated in cellular response to oxidative stress. Protein CoAlation was shown to modulate the activity, subcellular localization and regulatory interactions of modified proteins. Therefore, the development of CoAlation-specific antibodies, targeting the protein of interest, would provide important research tools for investigating functional importance of this modification in health and disease.

The advances in this emerging field of research will also require novel methodologies for measuring the stoichiometry of protein CoAlation, and quantifying changes in the level of CoA/CoA derivatives and enzymes involved in the CoAlation/deCoAlation cycle under various experimental conditions.

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Declaration of Competing Interest

The Authors declare that there are no competing interests associated with this manuscript.

Data availability

No data was used for the research described in the article.

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References

[1] R. Leonardi, Y.-.M. Zhang, C.O. Rock, S. Jackowski, Coenzyme A: back in action, Prog. Lipid Res. 44 (2005) 125–153.
[2] F.L. Theodoulou, O.C.M. Sibon, S. Jackowski, I. Gout, Coenzyme A and its derivatives: renaissance of a textbook classic, Biochem. Soc. Trans. 42 (2014) 1025–1032.
[3] O.C.M. Sibon, E. Strauss, Coenzyme A: to make it or uptake it? Nat. Rev. Mol. Cell Biol. 10 (2016) 605–606.
[4] Y. Tsuchiya, S.Y. Peak-Chew, C. Newell, S. Miller-Aidoo, S. Mangal, A. Zhyvoloup, J. Bakovic, O. Malanchuk, G.C. Pereira, V. Kotiadis, G. Szabadkai, M.R. Duchen, M. Campbell, S.R. Cuenca, A. Vidal-Puig, A.M. James, M.P. Murphy, V. Filonenko, M. Skehel, I. Gout, Protein CoAlation: a redox regulated protein modification by coenzyme A in mammalian cells, Biochem J 474 (2017) 2489–2508.
[5] I. Gout, Coenzyme A, protein CoAlation and redox regulation in mammalian cells, Biochem Soc Trans 46 (2018) 721–728.
[6] Y. Tsuchiya, A. Zhyvoloup, J. Baković, N. Thomas, B.Y.K. Yu, S. Das, C. Orengo, C. Newell, J. Ward, G. Saladino, F. Comitani, F.L. Gervasio, O.M. Malanchuk, A. I. Khoruzhenko, V. Filonenko, S.Y. Peak-Chew, M. Skehel, I. Gout, Protein CoAlation and antioxidant function of coenzyme A in prokaryotic cells, Biochem J 475 (2018) 1909–1937.
[7] I. Gout, Coenzyme A: a protective thiol in bacterial antioxidant defence, Biochem Soc Trans 47 (2019) 469–476.
[8] P.K. Tubbs, P.B. Garland, Variations in tissue contents of coenzyme A thioesters and possible metabolic implications, Biochem. J. 93 (1964) 550–557.
[9] C.M. Smith, C.R. Savage Jr, Regulation of coenzyme A biosynthesis by glucagon and glucocorticoid in adult rat liver parenchymal cells, Biochem. J. 188 (1980) 175–184.
[10] X. Liu, S. Sadhukhan, S. Sun, G.R. Wagner, M.D. Hirschey, L. Qi, et al., High-resolution metabolomics with acyl-CoA profiling reveals widespread remodelling in response to diet, Mol. Cell Proteomics 6 (2015) 1489–1500.
[11] Y. Wang, H. Yang, C. Geerts, A. Furtos, P. Waters, D. Cyr, S. Wang, G.A. Mitchell, The multiple facets of acetyl-CoA metabolism: energetics, biosynthesis, regulation, acylation and inborn errors, Mol Genet Metab 138 (2022), 106966.
[12] R.A. McAllister, L.M. Fixter, E.H.G. Campbell, The effect of tumour growth on liver pantothenate, CoA, and fatty acid synthetase activity in the mouse, Br. J. Cancer 57 (1988) 83–86.
[13] D.K. Reibel, B.W. Wyse, D.A. Berkich, J.R. Neely, Regulation of coenzyme A synthesis in heart muscle: effects of diabetes and fasting, Am. J. Physiol. 240 (1981) H606–H611.
[14] E.P. Brass, A.G. Tahiliani, R.H. Allen, S.P. Stabler, Coenzyme A metabolism in vitamin B-12 deficient rats, J. Nutr. 120 (1990) 290–297.
[15] B. Zhou, S.K. Westaway, B. Levinson, M.A. Johnson, J. Gitschier, S.J. Hayflick, A novel pantothenate kinase gene (PANK2) is defective in Hallervorden-Spatz syndrome, Nat. Genet. 28 (2001) 345–349.
[16] S. Dusi, L. Valletta, T.B. Haack, Y. Tsuchiya, P. Venco, S. Pasqualato, et al., Exome sequence reveals mutations in CoA synthase as a cause of neurodegeneration with brain iron accumulation, Am. J. Hum. Genet. 94 (2014) 11–22.
[17] A. Zhyvoloup, I. Nemazanyy, A. Babich, G. Panasyuk, N. Pobigailo, M. Vudmaska, V. Naidenov, O. Kukharenko, S. Palchevskii, L. Savinska, G. Ovcharenko, F. Verdier, T. Valovka, T. Fenton, H. Rebholz, M.L. Wang, P. Shepherd, G. Matsuka, V. Filonenko, Gout, I. Molecular cloning of CoA Synthase, The missing link in CoA biosynthesis, J Biol Chem 277 (2002) 22107–22110.
[18] M. Daugherty, B. Polanuyer, M. Farrell, M. Scholle, A. Lykidis, V. de Crecy-Lagard, A. Osterman, Complete reconstitution of the human coenzyme A biosynthetic pathway via comparative genomics, J. Biol. Chem. 277 (2002) 21431–21439.
[19] S. Aghajanian, D.M. Worrall, Identification and characterization of the gene encoding the human phosphopantetheine adenylyltransferase and dephospho-CoA kinase bifunctional enzyme (CoA synthase), Biochem. J. 365 (2002) 13–18.
[20] A. Zhyvoloup, I. Nemazanyy, G. Panasyuk, T. Valovka, T. Fenton, H. Rebholz, M. L. Wang, R. Foxon, V. Lyzogubov, V. Usenko, R. Kyyamova, O. Gorbenko, G. Matsuka, V. Filonenko, I. Gout, Subcellular localization and regulation of coenzyme A synthase, J Biol Chem 278 (2003) 50316–50321.
[21] J.D. Robishaw, D.A. Berkich, J.R. Neely, Rate-limiting step and control of coenzyme A synthesis in cardiac muscle, J. Biol. Chem. 257 (1982) 10967–10972.
[22] S. Skrede, O. Halvorsen, Mitochondrial biosynthesis of coenzyme A, Biochem. Biophys. Res. Commun. 91 (1979) 1536–1542.
[23] I. Nemazanyy, G. Panasyuk, A. Zhyvoloup, G. Panayotou, I.T. Gout, V. Filonenko, Specific interaction between S6K1 and CoA synthase: a potential link between the mTOR/S6K pathway, CoA biosynthesis and energy metabolism, FEBS Lett 578 (2004) 357–362.
[24] O. Breus, G. Panasyuk, I.T. Gout, V. Filonenko, I. Nemazanyy, CoA synthase is in complex with p85αPI3K and affects PI3K signaling pathway, Biochem. Biophys. Res. Commun. 385 (2009) 581–585.
[25] O. Breus, G. Panasyuk, I.T. Gout, V. Filonenko, I. Nemazanyy, CoA synthase is phosphorylated on tyrosines in mammalian cells, interacts with and is dephosphorylated by Shp2PTP, Mol. Cell Biochem. 335 (2010) 195–202.
[26] D. Gudkova, G. Panasyuk, I. Nemazanyy, A. Zhyvoloup, P. Monteil, V. Filonenko, et al., EDC4 interacts with and regulates the dephospho-CoA kinase activity of CoA synthase, FEBS Lett 586 (2012) 3590–3595.
[27] D.L. Martinez, Y. Tsuchiya, I. Gout, Coenzyme A biosynthetic machinery in mammalian cells, Biochem. Soc. Trans. 42 (2014) 1112–1117.
[28] J. Baković, D. López-Martínez, S. Nikolaou, B.Y.K. Yu, M.A. Tossounian, Y. Tsuchiya, C. Thrasivoulou, V. Filonenko, I. Gout, Regulation of the CoA biosynthetic complex assembly in mammalian cells, Int. J. Mol. Sci. 22 (2021) 1131.
[29] J. Olzhausen, T. Moritz, T. Neetz, H.J. Schüller, Molecular characterization of the heteromeric coenzyme A-synthesizing protein complex (CoA-SPC) in the yeast Saccharomyces cerevisiae, FEMS Yeast Res. 13 (2013) 565–573.
[30] G. Fiermonte, E. Paradies, S. Todisco, C.M.T. Marobbio, F. Palmieri, A novel member of solute carrier family 25 (SLC25A42) is a transporter of coenzyme A and adenosine 3',5'-diphosphate in human mitochondria, J. Biol. Chem. 284 (2009) 18152–18159.
[31] S.B. delCardayre, K.P. Stock, G.L. Newton, R.C. Fahey, J.E. Davies, Coenzyme A disulfide reductase, the primary low molecular weight disulfide reductase from Staphylococcus aureus. Purification and characterization of the native enzyme, J. Biol. Chem. 273 (1988) 5744–5751.
[32] O.M. Malanchuk, G.G. Panasyuk, N.M. Serbyn, I. Gout, V.V. Filonenko, Generation and characterization of monoclonal antibodies specific to Coenzyme A, Biopolym. Cell. 31 (2015) 187–192.
[33] Y. Tsuchiya, U. Pham, I. Gout, Methods for measuring CoA and CoA derivatives in biological samples, Biochem. Soc. Trans. 42 (2014) 1107–1111.
[34] L. Aloum, C.A. Brimson, A. Zhyvoloup, R. Baines, J. Baković, V. Filonenko, C.R. L. Thompson, I. Gout, Coenzyme A and protein CoAlation levels are regulated in response to oxidative stress and during morphogenesis in Dictyostelium discoideum, Biochem. Biophys. Res. Commun. 511 (2019) 294–299.
[35] A. Zhyvoloup, B.Y.K. Yu, J. Baković, M. Davis-Lunn, M.A. Tossounian, N. Thomas, Y. Tsuchiya, S.Y. Peak-Chew, S. Wigneshweraraj, V. Filonenko, M. Skehel, P. Setlow, I. Gout, Analysis of disulphide bond linkage between CoA and protein

cysteine thiols during sporulation and in spores of Bacillus species, FEMS Microbiol Lett. 367 (2020) fnaa174.
[36] J. Baković, B.Y.K. Yu, D. Silva, M. Baczynska, S.Y. Peak-Chew, A. Switzer, L. Burchell, S. Wigneshweraraj, M. Vandanashree, B. Gopal, V. Filonenko, M. Skehel, I. Gout, Redox regulation of the quorum-sensing transcription factor AgrA by Coenzyme A, Antioxidants 10 (2021) 841.
[37] S.-J. Reilly, V. Tillander, R. Ofman, S.E.H. Alexson, M.C. Hunt, The nudix hydrolase 7 is an Acyl-CoA diphosphatase involved in regulating peroxisomal coenzyme A homeostasis, J. Biochem. 144 (2008) 655–663.
[38] Y. Tsuchiya, D.P. Byrne, S.G. Burgess, J. Bormann, J. Baković, Y. Huang, A. Zhyvoloup, B.Y.K. Yu, S. Peak-Chew, T. Tran, F. Bellany, A.B. Tabor, A.E. Chan, L. Guruprasad, O. Garifulin, V. Filonenko, M. Vonderach, S. Ferries, C.E. Eyers, J. Carroll, M. Skehel, R. Bayliss, P.A. Eyers, I. Gout, Covalent Aurora A regulation by the metabolic integrator coenzyme A, Redox Biol 28 (2020), 101318.
[39] B.Y.K. Yu, M.A. Tossounian, S.D. Hristov, R. Lawrence, P. Arora, Y. Tsuchiya, S. Y. Peak-Chew, V. Filonenko, S. Oxenford, R. Angell, J. Gouge, M. Skehel, I. Gout, Regulation of metastasis suppressor NME1 by a key metabolic cofactor coenzyme A, Redox Biol. 44 (2021), 101978.
[40] J. Baković, B.Y.K. Yu, D. Silva, S.P. Chew, S. Kim, S.H. Ahn, L. Palmer, L. Aloum, G. Stanzani, O. Malanchuk, M.R. Duchen, M. Singer, V. Filonenko, T.H. Lee, M. Skehel, I. Gout, A key metabolic integrator, coenzyme A, modulates the activity of peroxiredoxin 5 via covalent modification, Mol Cell Biochem. 461 (2019) 91–102.
[41] D.A. Keire, J.M. Robert, D.L. Rabenstein, Microscopic protonation equilibria and solution conformations of coenzyme A and coenzyme A disulfides, J. Org. Chem. 57 (1992) 4427–4431, https://doi.org/10.1021/jo00042a022.
[42] D.M. Townsend, Y. Manevich, L. He, S. Hutchens, C.J. Pazoles, K.D. Tew, Novel role for glutathione S-transferase pi. Regulator of protein S-Glutathionylation following oxidative and nitrosative stress, J. Biol. Chem. 284 (2009) 436–445.
[43] S.G. Tajc, B.S. Tolbert, R. Basavappa, B.L. Miller, Direct determination of thiol pKa by isothermal titration microcalorimetry, J. Am. Chem. Soc. 126 (2004) 10508–10509.
[44] D.A. Keire, E. Strauss, W. Guo, B. Noszal, D.L. Rabenstein, Kinetics and equilibria of thiol/disulfide interchange reactions of selected biological thiols and related molecules with oxidized glutathione, J. Org. Chem. 57 (1992) 123–127.
[45] F.T. Ogata, V. Branco, F.F. Vale, L. Coppo, Glutaredoxin: discovery, redox defense and much more, Redox Biol. 43 (2021), 101975.
[46] L. Flohé, S. Toppo, L. Orian, The glutathione peroxidase family: discoveries and mechanism, Free Radic. Biol. Med. 187 (2022) 113–122.
[47] S.B. delCardayre, J.E. Davies, Staphylococcus aureus coenzyme A disulfide reductase, a new subfamily of pyridine nucleotide-disulfide oxidoreductase. Sequence, expression, and analysis of cdr, J. Biol. Chem. 273 (1998) 5752–5757.
[48] B.D. Wallace, J.S. Edwards, J.R. Wallen, W.J. Moolman, R. van der Westhuyzen, E. Strauss, M.R. Redinbo, A. Claiborne, Turnover-dependent covalent inactivation of Staphylococcus aureus coenzyme A-disulfide reductase by coenzyme A-mimetics: mechanistic and structural insights, Biochemistry 51 (2012) 7699–7711.