# Coenzyme A: a protective thiol in bacterial antioxidant defence

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**Key words:** coenzyme A (CoA), low molecular weight (LMW) thiols, reactive oxygen species (ROS), redox regulation in bacteria, post-translational modification, protein CoAlation.

**Abbreviations:** CoA, coenzyme A; GSH, glutathione; BSH, bacillithiol; mycothiol, MSH; LMW, low-molecular-weight; ROS, reactive oxygen species; NaOCl, sodium hypochloride; $H_2O_2$, hydrogen peroxide; SOD, superoxide dismutase; CAT, catalase; TRX, thioredoxin; GPX, glutathione peroxidase; CoADR, CoA disulfide reductase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; AhpC, alkyl hydroperoxide reductase C; TPX, thiol peroxidase; Mqo1/2, malate:quinone oxidoreductases 1 and 2; AgrA, accessory gene regulator A; PerR, peroxide operon regulator.

**Abstract**

Coenzyme A (CoA) is an indispensable cofactor in all living organisms. It is synthesised in an evolutionary conserved pathway by enzymatic conjugation of cysteine, pantothenate (Vitamin B5) and ATP. This unique chemical structure allows CoA to employ its highly reactive thiol group for diverse biochemical reactions. The involvement of the CoA thiol group in the production of metabolically active CoA thioesters (e.g. acetyl CoA, malonyl CoA, HMG CoA) and activation of carbonyl-containing compounds has been extensively studied since the discovery of this cofactor in the middle of last century. We are, however, far behind in understanding the role of CoA as a low-molecular-weight thiol in redox regulation. This review summarises our current knowledge of CoA function in redox regulation and thiol-protection under oxidative stress in bacteria. In this context, I discuss recent findings on a novel mode of redox regulation involving covalent modification of cellular proteins by CoA, termed protein CoAlation.

**Oxidative stress defences in bacteria**

Bacteria are adapted to grow and survive in enormously diverse conditions under aerobic or anaerobic environments. Obligate anaerobes cannot tolerate oxygen, whereas aerobes depend on aerobic respiration for growth and use oxygen as a terminal electron acceptor. While molecular oxygen provides substantial advantages in energy-yielding metabolism, it can readily react with other compounds resulting in the production of potentially damaging reactive oxygen species (ROS). In bacteria, the main sources of ROS include environmental redox reactions, redox-cycling antibiotics, hydrogen peroxide ($H_2O_2$) released by competing microbes, the oxidative burst of phagocytes, and endogenous ROS formed by enzyme autoxidation and redox enzymes [1,2]. The most common ROS are superoxide anion ($O^{2-}$), $H_2O_2$ and hydroxyl radical. ROS can exhibit positive or negative effects on biological processes in bacteria. They are implicated in regulatory signalling pathways and numerous physiological processes when present at low concentrations in cells. However, high levels of ROS that overwhelm cellular antioxidant defence systems promote oxidative stress, which may have deleterious effects on essential cellular functions and ultimately lead to cell death [3-5].

To sense and combat oxidative stress and repair the subsequent damage, bacteria employ various defence mechanisms including prevention of ROS production, expression of redox-sensing factors, ROS detoxification enzymes, low-molecular-weight (LMW) thiols, and activation of oxidative damage repair pathways (Fig. 1). A diverse range of regulatory sensors continuously monitor the redox state of internal and external environments and coordinate regulatory pathways that function to maintain the redox homeostasis.

Sensing mechanisms are diverse and may involve basic sensory units in the context of regulatory proteins, such as iron and iron–sulphur clusters, haem centres, flavins, pyridine nucleotides and the sulphur-containing amino acids cysteine (Cys) and methionine [6,7]. Solvent-exposed cysteine residues are commonly employed in redox sensing and signalling processes, because the thiol side-chain can be oxidized to several reversible and irreversible redox states, including sulphenic acid (R-SOH), sulphinic acid (R-$SO_2$H), sulphonic acid (R-$SO_3$H), and S-nitrosothiol (R-SNO). In bacteria, redox sensors usually function at the level of transcription, allowing for a fast response to the altered redox environment by upregulating specific pathways involved in the detoxification of reactive species and the repair of oxidative damage. Redox-sensing transcription factors, such as OxyR and SoxR/S in *E. coli* and PerR, sigmaB and OhrR in Gram-positive bacteria, are recognized as the principle regulators of the oxidative stress response in a broad spectrum of bacterial species [8].

Bacterial cells activate innate antioxidant defence systems when exposed to exogenous or endogenously produced ROS, that involve scavenging enzymes and LMW thiols. To remove excess of O2− and $H_2O_2$, bacteria express dedicated scavenging enzymes that neutralize harmful oxidants before they cause damage to cellular components, including DNA, membrane lipids and proteins [4, 9]. Superoxide dismutases (SOD), catalases (CAT), thioredoxins (TRX) and glutathione peroxidases (GPX) are the key enzymatic antioxidants in bacteria. Bacteria also produce high levels of LMW thiols that serve as redox buffers and provide protection against ROS. The most common LMW thiols in bacteria include glutathione (GSH), cysteine (Cys), bacillithiol (BSH), mycothiol (MSH) and CoA (Fig. 2) [10-13]. They are structurally diverse and exhibit different expression profiles, biophysical and biochemical properties, and cellular functions [14]. One common feature of these redox active LMW thiols is that their -SH functional group originates from cysteine. In contrast to eukaryotic cells, bacteria lack membrane-bound organelles such as mitochondria and endoplasmic reticulum, in which the redox state is optimized for specialised cellular processes. The cytoplasm of bacterial cells is a reducing environment, which allows protein thiols to maintain their reduced state. Bacteria actively employ LMW thiols as their intracellular redox buffer, and thiol-disulphide oxidoreductases to maintain a reduced state of the cytoplasm. To effectively detoxify different reactive oxygen species, LMW thiol-redox buffers are produced at high levels and often present in millimolar concentrations in bacterial cytoplasm. Differential production of LMW thiols is observed in bacteria. Among major LMW thiols, Cys and CoA are ubiquitously produced in all bacterial species. GSH is present in

millimolar concentrations in Gram-negative and only few Gram-positive bacteria, while the production of BSH and MSH is restricted to Firmicutes and Actinomycetes respectively. The standard redox potential of major LMW thiols varies from - 221 mV (BSH) to - 240 mV (GSH), which implies that they exhibit different capacities to buffer oxidative stress (Fig. 2) [15-18]. They also have relatively high pKa values (7.97 for BSH and 9.83 for CoA), which protects their thiol groups from converting to the sulphenic acid state under oxidative stress [17-20].

LMW thiols can also covalently modify protein Cys residues in a process called S-thiolation, which can occur via ROS dependent or ROS independent mechanisms [21]. The ROS-independent mechanism of protein S-thiolation may occur when nitrosothiols (RSNO) are generated during nitric oxide (NO) metabolism or when cells are exposed to non-oxidizing and very electrophilic compounds (e.g. diamide). ROS-dependent protein S-thiolation is initiated when Cys residues are oxidized to a highly reactive sulphenic acid or via the disulphide-exchange and radical reactions.

Protein S-glutathionylation is the most important and best studied post-translational thiol modification in mammalian cells and bacteria, while recent advances in mass spectrometry allowed the identification of other forms of S-thiolation, including S-bacillithiolation, S-mycothiolation and S-CoAlation [22-25].

**Coenzyme A: a key metabolic integrator and a protective thiol in bacteria**

CoA is a ubiquitous and essential cofactor in all living organisms. Fritz Lipmann discovered CoA in the middle of last century and showed that it is a fundamental catalytic substance in cellular conversion of nutrients into energy and an important player in intermediary metabolism [11]. Lipmann received the Nobel prize for this discovery which he shared with Hans Krebs, who was honored for his discovery of the citric acid cycle. CoA is synthesized in an evolutionary conserved pathway that requires pantothenate (vitamin B5), cysteine and ATP [26]. The presence of a highly reactive thiol group and a nucleotide moiety in the CoA structure are at the core of its cellular functions. This unique chemical structure allows CoA to activate carbonyl-containing molecules in catabolic and anabolic reactions, and to bind a diverse range of carboxylic acids, resulting in the formation of metabolically active thioester derivatives, such as acetyl CoA, malonyl CoA, 3-hydroxy-3-methylglutaryl CoA, acyl CoA etc. CoA and its thioesters are widely implicated in central metabolic pathways, including the citric acid cycle, fatty acid biosynthesis and oxidation, amino acid metabolism, isoprenoid and peptidoglycan biosynthesis and others (Fig. 3A) [27-29].

Redox reactions are central to both anabolic and catabolic metabolism, and therefore balancing the redox state is vital for optimal bacterial metabolism, growth and survival. LMW thiols play an essential role in the maintenance of a reducing environment in the cytosol and protection of bacteria against external threats. While significant progress has been made to understand the role of the CoA thiol group in the production and function of various thioester derivatives and activation of carbonyl-containing groups, the involvement of the CoA thiol moiety in redox regulation and antioxidant defence remains largely unknown.

The distribution, abundance, biochemical and biophysical properties of LMW thiols are key factors that determine their potential to function as redox buffers and protect bacteria against oxygen toxicity. Furthermore, the level of expression and catalytic properties of enzymes implicated in diverse thiol-specific detoxification and disulphide-reducing pathways control the efficacy of LMW thiols in redox regulation and cellular stress response.

There is a limited body of research literature on the size and composition of the intracellular CoA pool in bacteria. Published studies reported that CoA is present in bacteria at relatively high levels where the total level of CoA varied from 0.4 mM in *E. coli*, to low millimolar level in *S. aureus* [30-32]. The abundance and composition of the intracellular pool of CoA depends on the cellular redox state regulated/controlled by the availability of nutrients as well as exposure to stress conditions. Analysis of CoA species in growing *E. coli* showed that the level of free CoA (13.8%) is significantly lower when compared to acetyl CoA (79.8%) [31]. These findings are in agreement with the widely

acknowledged central role of acetyl CoA and other metabolically active CoA thioesters in the regulation of cell growth and division. In contrast, culturing bacteria in glucose-deprived medium resulted in the depletion of CoA thioesters, while free CoA became the major component (82%) of the CoA pool (31). One may therefore speculate that the increase in the level of free CoA under stress conditions may allow bacteria to sense, respond and adapt to excessive ROS accumulation. Recently, new methods for accurate and reliable measurement of CoA species in biological samples have been developed [33,34]

A key property of CoA as a LMW thiol is its resistance to autoxidation. The thiol group position in the CoA structure determines its high pKa and low potential for the chelation of a catalytic metal which would stabilise it for autoxidation. When compared to other LMW thiols, copper-catalyzed air oxidation of CoA occurs at a rate which is 4-fold slower than GSH and 720-fold slower than cysteine [35]. The intrinsic pKa value for the CoA thiol-thiolate equilibrium is high (9.83), indicating that CoA exists predominantly in its unreactive thiol form at physiological pH [20]. In B. subtilis, the percentages of thiolate forms of Cys, BSH and CoA at physiological pH were found to be 15, 22 and 1%, respectively [36]. The low level of CoA thiolate and abundance of CoA thiol in B. subtilis most probably account for the weak reactivity of CoA with different electrophilic biomolecules. To perform a nucleophilic attack, the CoA thiol needs to be activated to a thiolate state. The mechanism of activation may involveenzyme(s) which can reduce the pKa value of the CoA thiol and facilitate covalent modification of cellular targets, as reported for GSH in complex with glutathione S-transferase-pi (GST-pi) [37,38]. The relatively high pKa of the CoA thiol also protects it from oxidation to the sulphenic acid state (CoASOH) [20]. The standard redox potential of CoA (-234 mV) is close to that of GSH (-240 mV), which implies it has a good capacity to buffer oxidative stress [17]. Insights into the role of CoA in redox regulation in bacteria came from the identification of CoA disulphide reductase (CoADR) in *S. aureus* and later on in other bacteria [39]. These original findings provided the foundational support for the existence of a CoA-based thiol/disulphide redox system in bacteria involved in resisting oxidative stress and maintaining the reducing environment in cells.

**Redox regulation and protein CoAlation**

Protein CoAlation has recently emerged as a major and widespread post-translational thiol modification in cellular response to oxidative and metabolic stress (Fig. 3B) [25]. There might be several reasons why it took so long to uncover the redox-regulated covalent protein modification by CoA. First of all, extensive research on cellular functions of CoA thioesters and the role of CoA in the catalysis of catabolic and anabolic reactions overshadowed several biochemical and crystallographic studies which reported the formation of mixed disulphides between CoA and cysteine residues of specific proteins [40-43]. Secondly and more importantly, the lack of specific research tools and methodologies hampered proteome-wide analysis and identification of CoA-modified proteins under various experimental conditions, including oxidative stress. CoA is a weak antigen, and anti-CoA antibodies are not yet commercially available. The development of a highly specific anti-CoA monoclonal antibody, which recognises CoA in ELISA, Western blotting, immunoprecipitation and immunohistochemistry has provided a powerful tool for advancing the knowledge on CoA biology in health and disease [44]. It has been expertly applied by two research consortiums for uncovering extensive redox-induced modification of cellular proteins by CoA in eukaryotic and prokaryotic cells [45,46]. The availability of anti-CoA antibody which works efficiently in immunoprecipitation allowed for the development of a robust mass spectrometry-based methodology for the identification of CoAlated proteins [45].

Protein CoAlation was originally reported in mammalian cells and tissues exposed to oxidative or metabolic stress [45]. This study has provided a foundation for investigating the extent and relevance of protein CoAlation in other model organisms, including yeast, amoeba and bacteria. Examining the role of CoA in bacterial redox regulation and adaptation to stresses was of particular interest, since other LMW thiols exhibit thiol reactivity profiling and production levels, especially in Gram-positive

bacteria [30]. Recently, convincing evidence was provided demonstrating that protein CoAlation occurs at a basal level in exponentially growing Gram-negative (*E.coli*) and Gram-positive bacteria (*S. aureus* and *B. megaterium*), while exposure to thiol-oxidizing agents, such as $H_2O_2$, diamide and sodium hypochloride NaOCl, induces extensive covalent protein modification by CoA in a DTT-sensitive manner [46]. It is well-established that nutrient deprivation is associated with ROS production and oxidative stress. When protein CoAlation was examined in a model of glucose deprivation, a significant increase in the level of CoA-modified proteins was observed in Gram-negative and Gram-positive bacteria cultured in the absence of glucose as the carbon source. The reversible nature of protein CoAlation was demonstrated in both experimental setups, when bacteria were allowed to recover from oxidative and metabolic stress by the removal of oxidising agents or the re-addition of glucose to the culture medium [46].

The mass spectrometry-based methodology revealed the identity of 356 proteins in *S. aureus* (over 12% of gene products), which were CoAlated in response to diamide-induced stress [46]. Bioinformatics analysis showed that proteins involved in cellular metabolism, antioxidant response, regulation of transcription and translation are the main targets of CoAlation. Similarly to mammalian redox-induced CoAlome, the majority of CoA-modified proteins in diamide-treated *S. aureus* are involved in metabolic processes (68%), suggesting the importance of CoA in the regulation of cellular metabolism under oxidative stress. Key players of the citric acid cycle, glycolysis, gluconeogenesis, glycerol catabolism and the glyoxylate shunt are in the list of CoAlated proteins.

Regulators of transcription form the second largest functional group of CoA-modified proteins (7%), which was not the case in mammalian cells. Among identified proteins, there are redox-sensing transcriptional regulators, including ArgA, CtsR, PerR, SarR and SarS, which control global gene expression via the redox-active cytsteine residues. The effect of CoAlation on their DNA-binding and transcriptional activities in response to oxidative and metabolic stress remains to be investigated. The identification of many ribosomal proteins and regulators of translation among CoAlated proteins may suggest the inhibitory effect of this modification on protein synthesis under oxidative stress.

The third largest group of CoAlated proteins in diamide-treated *S. aureus* comprises regulators of stress response. Several important antioxidant proteins are among the targets of CoAlation, including thioredoxin (Trx), alkyl hydroperoxide reductase C (AhpC), thiol peroxidase (Tpx), malate:quinone oxidoreductases 1 and 2 (Mqo1/2), and Fe–S oxidoreductase (YtqA). In case of Tpx, diamide-induced CoAlation involves catalytic Cys60 and the relevance of this modification is yet to be investigated.

The question which arises from these original findings is: what is the relevance of CoAlation in the regulation and function of modified proteins? CoA is a bulky (767 Da) and charged molecule, so covalent modification of targeted proteins alters their molecular weight and charge, and has the potential to modulate their stability, enzymatic properties, subcellular localization and regulatory interactions (Fig. 4). Identification of CoA-modified cysteines in metabolic enzymes and transcription factors, and the development of an efficient in vitro CoAlation assay allowed to study the effect of CoAlation on their enzymatic and transcriptional activities [46 and Bakovic et al., unpublished studies]. For example, in vitro CoAlation of *S. aureus* glyceraldehyde-3-phosphate dehydrogenase was shown to inhibit its enzymatic activity and protect the catalytic Cys151 from overoxidation by $H_2O_2$, providing a reversible mode for regeneration of this essential glycolytic enzyme during the recovery from oxidative stress. CoAlation may also generate a unique binding motif for intra- and intermolecular regulatory interactions, mediated via the pantetheine tail and/or the 3′,5′-ADP moiety.

**Conclusions and future perspectives**

Protein CoAlation is an emerging field of research. Therefore, understanding the molecular mechanisms of the protein CoAlation/deCoAlation cycle will be the main challenge in the coming years. The forward reaction can be achieved through a thiol-disulphide

mechanism or enzymatic conjugation of CoA to protein cysteine thiols mediated by a currently unknown CoA transferase (analogous to GST-pi). It remains to be determined whether the induction

of protein CoAlation under oxidative or metabolic stress is accompanied by the increase in the CoASSCoA level and a subsequent decrease in the CoASH/CoASSCoA redox ratio in examined bacteria.

To participate in the maintenance of protein thiols in their reduced state, CoA should function in concert with dedicated enzymes, possessing CoADR and CoAredoxin activities. CoADR was identified in many bacterial species, but its role in the mechanism of protein deCoAlation remains to be investigated. The identity and specificity of CoAredoxin enzymes implicated in protein deCoAlation will be the subject of future studies.

To further advance the research on protein CoAlation and anti-oxidant function of CoA it is important to develop new research tools and methodologies. The priority lies in developing reliable methods for quantifying changes in CoA and related metabolites under different conditions, and for the quantitative measurement of protein CoAlation in proteome-wide studies

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**Acknowledgements**
I thank the members of Cell Regulation Laboratory at the Department of Structural and Molecular Biology (UCL) and collaborators on the protein CoAlation project for their valuable inputs. I am grateful to J. Bakovic and R. O'Sullivan for critical reading of the manuscript.

**Funding**
University College London Business [13-014 and 11-018] and the Biotechnology and Biological Sciences Research Council [BB/L010410/1].

**Declarations of interests**
The Author declares that there are no competing interests associated with the manuscript.

**Figures:**

**Figure 1.** Antioxidant defense mechanisms in bacteria
**Figure 2.** Structure and chemical properties of major low-molecular weight (LMW) thiols in bacteria. Original studies describing the identification of LMW thiols are shown in parentheses. The pKa values and redox potential are presented.
**Figure 3.** The role of the CoA thiol group in cellular metabolism and redox regulation
**Figure 4.** Protein CoAlation and its cellular functions.

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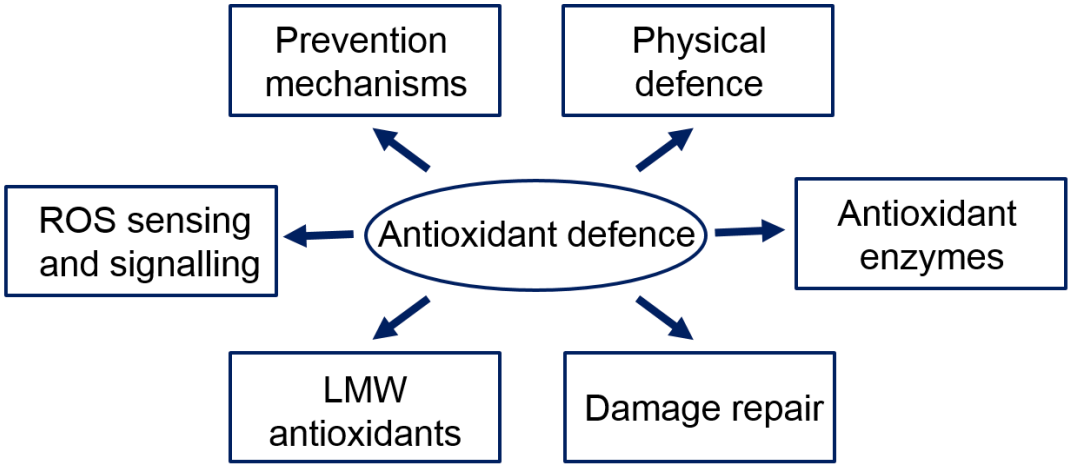
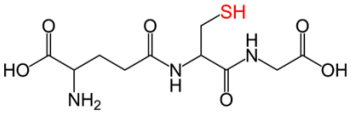
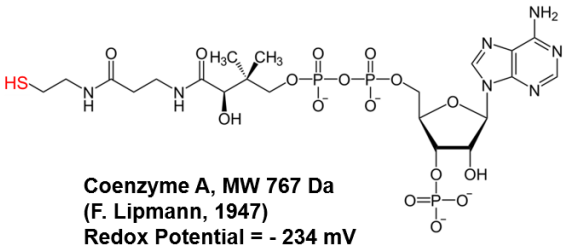

Prevention mechanisms
Physical defence
ROS sensing and signalling
Antioxidant defence
Antioxidant enzymes
LMW antioxidants
Damage repair

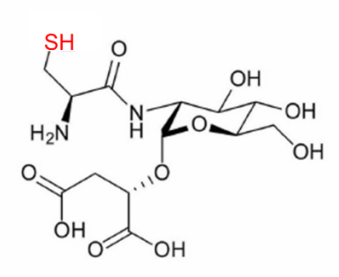
Figure 1



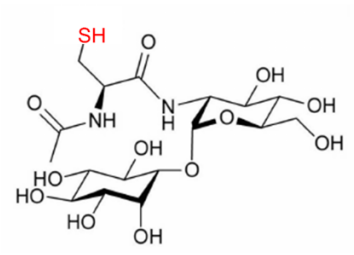
**Glutathione, MW 380 Da**
**(F. Hopkins, 1929)**
**Redox Potential = - 240 mV**
**and $pK_a$ 8.93 [15,19]**



**Coenzyme A, MW 767 Da**
**(F. Lipmann, 1947)**
**Redox Potential = - 234 mV**
**and $pK_a$ 9.83 [16,20]**



**Bacillithiol, MW 398 Da**
**(G. Newton et al., 2009)**
**Redox Potential = -221 mV,**
**and $pK_a$ = 7.97 [17]**



**Micothiol, MW 486 Da**
**(G. Newton et al., 1993)**
**Redox Potential = -230 mV**
**and $pK_a$ 8.76 [18]**

Figure 2

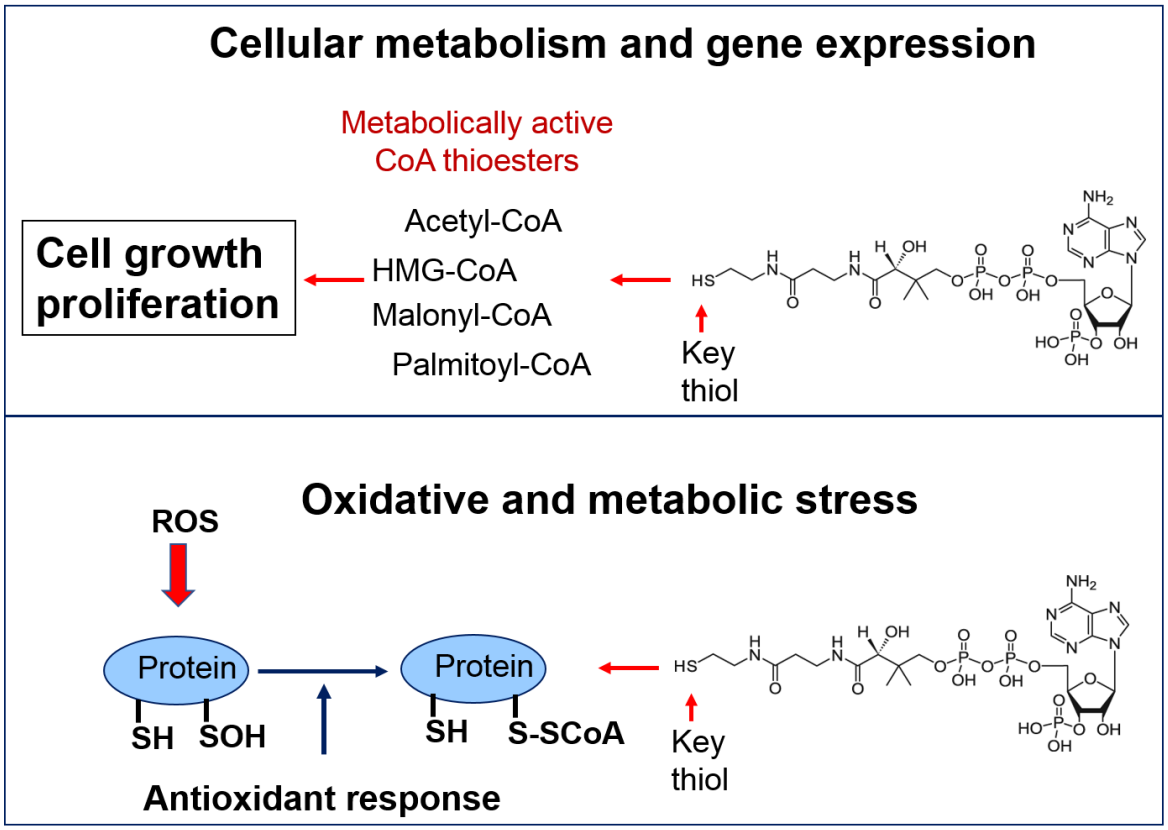

Cellular metabolism and gene expression
Metabolically active
CoA thioesters
Cell growth
proliferation
Acetyl-CoA
HMG-CoA
Malonyl-CoA
Palmitoyl-CoA
Key
thiol
Oxidative and metabolic stress
ROS
Protein
SH
SOH
Protein
SH
S-SCoA
Key
thiol
Antioxidant response

Figure 3