

Review

Acetyl-coenzyme A synthetase (AMP forming)

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Abstract. Acetyl-coenzyme A synthetase (AMP forming; Acs) is an enzyme whose activity is central to the metabolism of prokaryotic and eukaryotic cells. The physiological role of this enzyme is to activate acetate to acetyl-coenzyme A (Ac-CoA). The importance of Acs has been recognized for decades, since it provides the cell the two-carbon metabolite used in many anabolic and energy generation processes. In the last decade researchers have learned how carefully the cell monitors the synthesis and activity of this enzyme. In eukaryotes and prokaryotes,

complex regulatory systems control *acs* gene expression as a function carbon flux, with a second layer of regulation exerted posttranslationally by the NAD⁺/sirtuin-dependent protein acetylation/deacetylation system. Recent structural work provides snapshots of the dramatic conformational changes Acs undergoes during catalysis. Future work on the regulation of *acs* gene expression will expand our understanding of metabolic integration, while structure/function studies will reveal more details of the function of this splendid molecular machine.

Key words. Two-carbon metabolism; acetyl-coenzyme A; acetate; CoA ligase; lifespan; gene expression; sirtuins; chromosome stability; gene silencing.

Introduction

In nature, acetate is a very abundant short-chain fatty acid that eukaryotes and prokaryotes alike use in many of their metabolic processes. In environments as diverse as soil and the gastrointestinal tract of animals, including humans, the concentration of acetate can reach very high levels [1–3]. All cells activate acetate before they metabolize it. Prokaryotic cells have evolved three distinct pathways to convert this short-chain fatty acid into acetyl-coenzyme A (Ac-CoA) (fig. 1). By combining the use of these pathways, prokaryotes can use acetate as a carbon and energy source regardless of its concentration in the environment. One pathway comprises the acetate kinase (AckA, EC 2.7.2.1)/phosphotransacetylase (Pta,

EC 2.3.1.8) enzymes, which activate acetate to Ac-CoA via Ac-phosphate (Ac-P) (fig. 1A) [4]. The Ack/Pta system is the route used by prokaryotes when acetate is present in high concentrations in the environment (≥ 30 mM acetate). Many fermentative and facultative anaerobic bacteria take advantage of the reversibility of the Ack/Pta system to conserve energy and maintain steady-state levels of free CoA in the cell. For this purpose, cells use Pta to convert Ac-CoA to Ac-P, which is consumed by Ack in a reaction that yields ATP and acetate [5, 6]. Although Ac-P is widely used to conserve energy, it is also involved in the regulation of gene expression by two-component regulatory systems [7–11]. A second pathway of acetate activation to acetyl-CoA is composed of Ac-CoA synthetase (ADP forming) and a third route is composed of Ac-CoA synthetase (AMP forming). Acetyl-CoA synthetase (ADP forming, EC 6.2.1.1.3) catalyzes the re-

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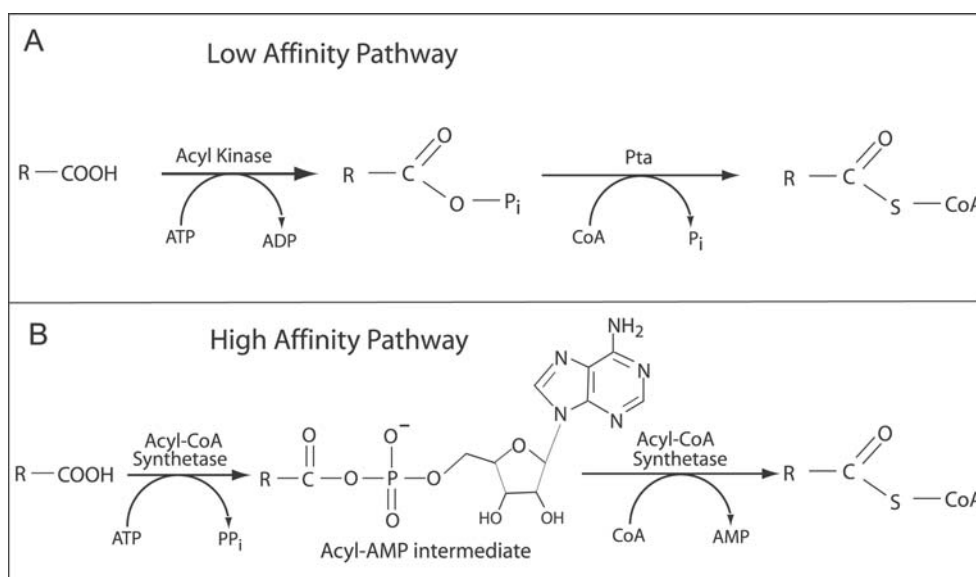


Figure 1. Pathways for the conversion of acetate to ac-CoA. Pta, phosphotransacetylase. Affinity is defined by the concentration of acetate in the medium that is optimally used by the indicated pathway. The low-affinity pathway is optimally functional acetate concentrations ≥ 30 mM; < 10 mM acetate for the high-affinity pathway.

versible reaction $\text{acetate} + \text{ATP} \rightarrow \text{ADP} + \text{P}_i + \text{Ac-CoA}$. This type of enzyme was first discovered in the eukaryotic parasite *Entamoeba histolytica* and the anaerobic protozoan *Giardia lamblia*, where it is required for ATP synthesis during fermentative growth [12–14]. In prokaryotes, ADP-forming Ac-CoA synthetases were first described in detail in the archaeon *Pyrococcus furiosus* [15, 16]. In this and other members of the Archaea domain, this enzyme catalyzes the formation of acetate from Ac-CoA, driving the phosphorylation of ADP to yield ATP. ADP-forming Ac-CoA synthetases belong to the class of acyl-CoA synthetase enzymes that catalyze the synthesis of product in a single step. Structural studies of succinyl-CoA synthetase (EC 6.2.1.5) provided valuable insights into the mechanism of function of the enzyme by establishing the oligomeric state of the active enzyme ($\alpha_2\beta_2$ dimer-of-dimers tetramer), the presence of a phosphorylated histidyl residue at position 246 located in close proximity to the thiol group of CoA, the location of the CoA and NTP binding sites and the mode of binding of CoA [17, 18]. Unlike the ADP-forming Ac-CoA synthetases, AMP-forming Ac-CoA synthetases (ATP-dependent acetate:CoA ligase, EC 6.2.1.1, Acs) synthesize Ac-CoA in two steps (fig. 1B) via a different mechanism (see below). Acs is the preferred route of Ac-CoA synthesis when the concentration of acetate in the environment is low (≤ 10 mM acetate). In eukaryotes, the role of Acs is more critical than in prokaryotes since it is the only route for the activation of acetate to Ac-CoA. This review focuses on the regulation and function of AMP-forming Ac-CoA synthetases and the regulation of

the genes encoding these enzymes in prokaryotes and eukaryotes.

The AMP-forming family of enzymes

AMP-forming enzymes catalyze the synthesis of product via an acyl-adenylate (acyl-AMP) intermediate, and are involved in many catabolic and anabolic processes. Scientists from many disciplines (e.g. enzymologists, structural biologists, molecular biologists, cell physiologists, natural products chemists) have studied this family of enzymes because of their involvement in the synthesis of antibiotics, anti-cancer agents and the degradation of pollutants [19–22]. The half-reactions catalyzed by AMP-forming enzymes are shown in figure 1B. X-ray crystallography has provided three-dimensional views of the active site of these enzymes (see below).

The AMP-forming acetyl-CoA synthetase (Acs) enzyme is conserved in nature

The *acs* gene is ubiquitous in nature. Acs orthologs are found in bacteria, in some thermophilic and extremely thermophilic archaea, and in eukaryotes, including fungi, plants and humans [23–32].

Bioinformatics analyses suggest that all Acs orthologs share a common ancestor. Results of phylogenetic analyses based on 30 protein sequences suggest a prokaryotic origin for eukaryotic Acs enzymes [33]. A comparison of

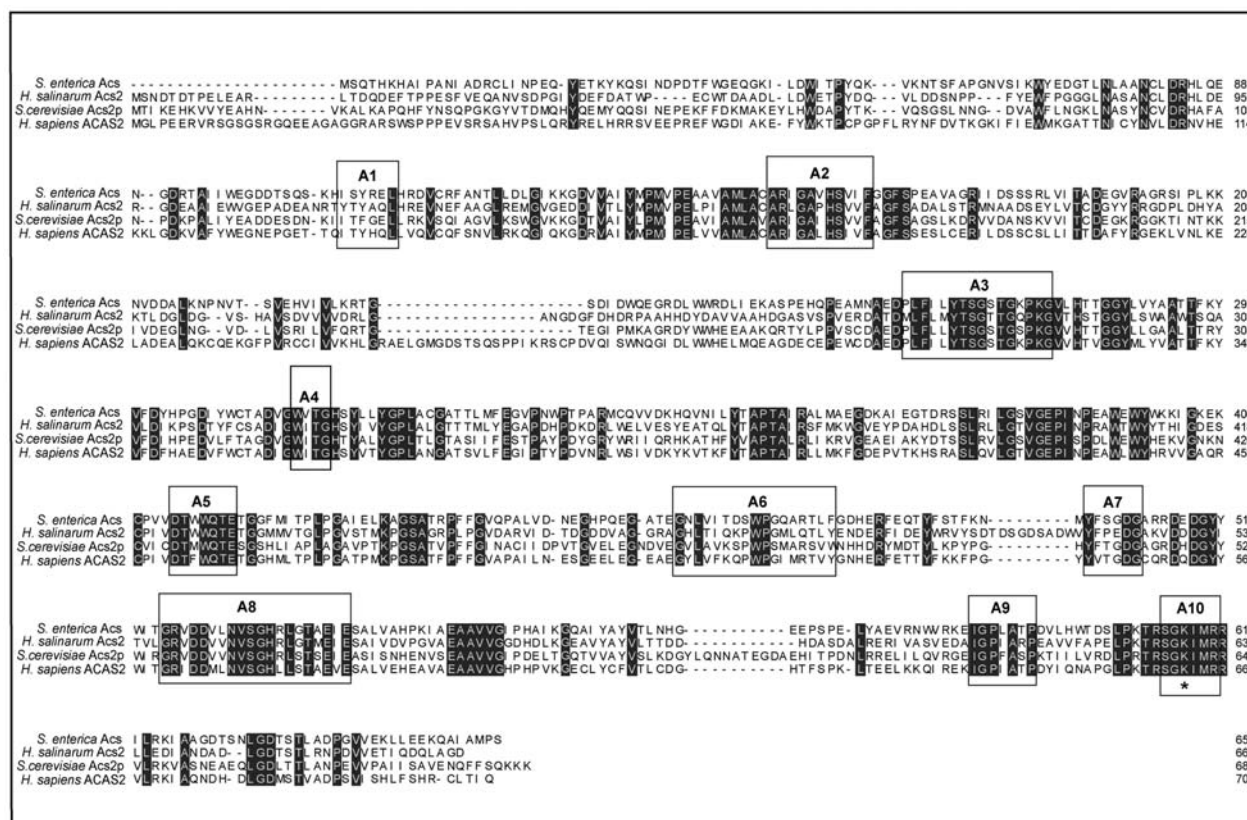


Figure 2. Homology amongst ac-CoA synthetases from representative organisms from all domains of life. *Salmonella enterica*, a γ -proteobacterium; *Halobacterium salinarum*, an extremely halophilic archaeon; *S. cerevisiae*, yeast; *Homo sapiens* (humans). Sequence motifs conserved amongst members of the AMP-forming family of enzymes are boxed and labeled A1 through A10. The asterisk identifies the lysyl residue of the A10 domain that is reversibly acetylated.

the primary amino acid sequences of Acs enzymes from representatives of all three domains of life is shown in figure 2. There are many conserved regions throughout the length of the Acs proteins, which are approximately the same size (~70 kDa). Such conservation of size suggests that extant Acs is probably the minimal structure required for the catalysis of the two half-reactions (fig. 1B), and that the need for a larger enzyme has not arisen in nature.

Synthesis of acetyl-CoA by AMP-forming acetyl-CoA synthetase

Acs catalyzes the synthesis of Ac-CoA via an ordered Bi Uni Uni Bi ping-pong mechanism. The enzyme binds ATP first, then acetate, leading to the formation of enzyme-bound acetyl-AMP and the release of pyrophosphate. Ac-CoA is formed after CoA binds to Acs, with the reaction ending with the sequential release of Ac-CoA and AMP [34–40].

Our understanding of how Acs synthesizes Ac-CoA is incomplete. Glimpses of how this enzyme works were revealed by kinetic, stereochemical, and structural analyses

of wild-type and mutant Acs and other AMP-forming enzymes isolated from diverse organisms.

The first half-reaction: acetate activation to acetyl-AMP

The adenylation step occurs with inversion of configuration at the α -phosphorus of ATP. Support for this conclusion comes from studies of the stereochemical course of acetate activation using [^{18}O]acetate and adenosine 5'-(1-thio)-triphosphate diastereoisomers A and B [43]. Under the conditions used, only the B isomer of ATP [αS] [41, 42] was used by the yeast Acs enzyme, which incorporated labeled oxygen in opposite orientation from the leaving pyrophosphate group. When the labeled product was converted back to ATP[αS], the amount of labeled oxygen was almost entirely (98.5%) located at the position occupied by the oxygen bridging the α and β phosphorus atoms. This implies that the entering and leaving groups (acetate and pyrophosphate, respectively) are in opposed, in-line orientation relative to each other, and that a single displacement event leads to acetyl-AMP formation [43].

X-ray crystallography has provided insights into the fold of AMP-forming enzymes and into the conformations adopted by these proteins during the catalysis of the first and second half-reactions [44–49]. Three-dimensional crystal structures reveal that AMP-forming enzymes have a large N-terminal domain (>500 residues) and a small (~110 residues) C-terminal domain. In these enzymes, the active site is at the domain interface, and its contents determine the orientation of the C-terminal domain. In the structure of unliganded luciferase, the C-terminal domain is in an open conformation, while the structure of PheA (the adenylation domain of gramicidin synthetase 1) liganded to phenylalanine and AMP shows the C-terminal domain is rotated 90° relative to that of luciferase [47, 48].

A model of the three-dimensional structure of the yeast ACS1 enzyme complexed with AMP was published during the writing of this review [45]. The first feature to note is that unlike the bacterial Acs, yeast Acs is a stable trimer (PDB code 1RY2). This model of yeast Acs reveals the conformation of the enzyme that is likely to catalyze the first half-reaction, i.e., the synthesis of acetyl-AMP. In this model, the conserved lysyl residue in the conserved A10 motif (marked with an asterisk in fig. 2) is in close proximity to AMP, but the electron density of the side chain is missing. In the structure of PheA complexed with phenylalanine and AMP, the side chain of the A10 Lys is hydrogen bonded to the oxygen of the ribose ring that bridges the ribose and α -phosphate, and to the carbonyl oxygen of phenylalanine. These structures suggest an important role for the A10 Lys residue in orienting one or both substrates during the first half-reaction. In the closely related propionyl-CoA synthetase, a lysine-to-

glutamate change at residue Lys592 (the A10 Lys) reduced the specific activity of the enzyme by >4 orders of magnitude when propionate, ATP and CoA were used as substrates. Interestingly, the same mutation did not affect the thioester-forming activity of the enzyme [50], suggesting this residue is not involved in the catalysis of the second half-reaction. Other residues of Acs critical to the formation of the acetyl-AMP intermediate were identified through the kinetic analysis of variants of *Bradyrhizobium japonicum* Acs [51]. From these studies the authors conclude that residues Gly266 and Lys269 are involved in the formation of acetyl-AMP, with residue Glu414 being important in binding acetate. We found discrepancies in the numbering of residues between the above-mentioned work and the NCBI database, and thought the reader should be aware of them. A major discrepancy is the length of the protein. The *B. japonicum* gene annotated as *acs* predicts a protein 636 amino acids in length, which is different than the reported 648-amino acid enzyme. Probably because of the differences in length, residue numbering is off. The numbering problem does not appear to be related to strain differences.

The second half-reaction: conversion of acetyl-AMP to acetyl-CoA

How Acs catalyzes the second half-reaction is poorly understood. A model of the three-dimensional structure of a bacterial Acs enzyme complexed with CoA and an alkyl-AMP inhibitor is available [44]. In this model, the Acs enzyme is in the conformation that is likely to catalyze the second half-reaction (figs. 3, 4), hence it provides the framework for dissecting the mechanism of thioester

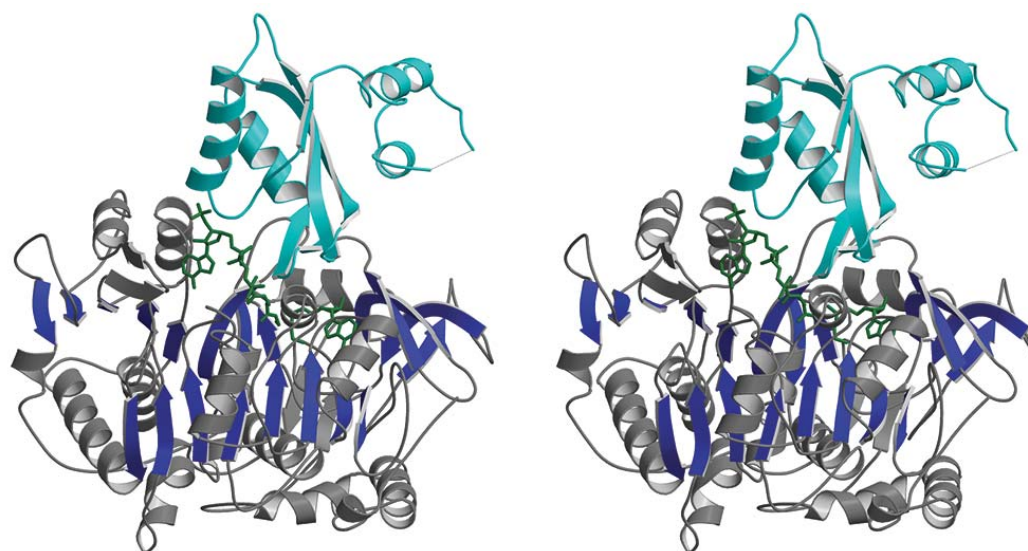


Figure 3. Structure of *S. enterica* Acs. Stereo view of the ribbon representation of *S. enterica* Acs complexed with CoA and adenosine-5'-propylphosphate [44]. The β sheets that form the N-terminal domain are shown in blue. The binding sites for the two ligands are indicated by ball-and-stick figures. The C-terminal domain is shown in light blue-green color. PDB code 1pg4.

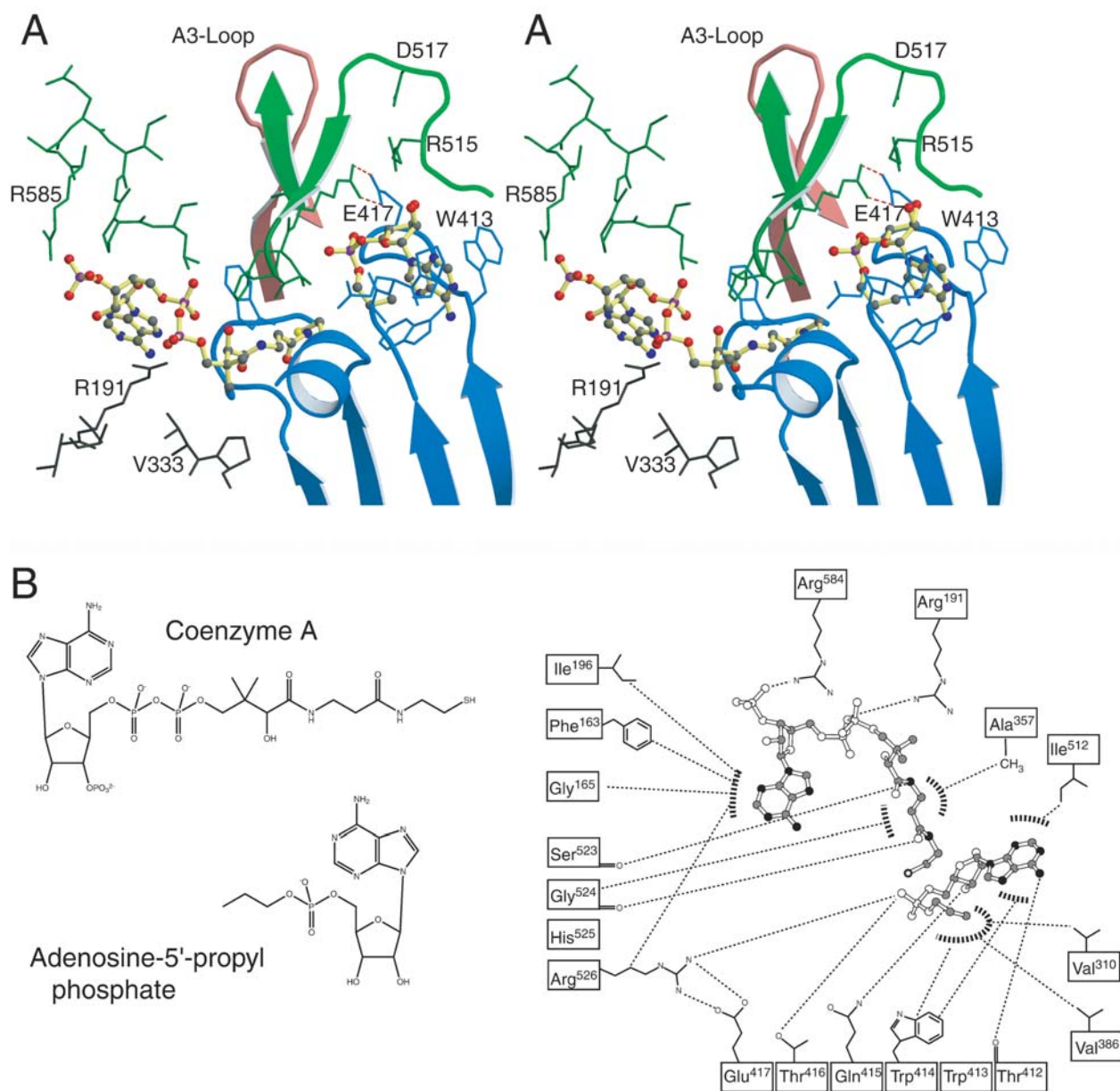


Figure 4. Detailed view of the interactions of Acs with the CoA and adenosine-5'-propylphosphate. (A) Stereorepresentation of the binding pocket showing the surface-binding portion of the nucleotide portion of CoA. Ligands are represented by ball-and-stick structures with yellow bonds. The phosphopantetheine moiety of CoA is directed into the adenylate-binding pocket. The glycine-rich A3 motif [19] is shown in pink. (B) Protein residues that interact with the ligands are shown. Dashed lines show the interactions between different functional groups. Hydrophobic interactions are indicated by broad hashed curves. Reproduced with permission from *Biochemistry* (2003) **42**: 2866–2873. Copyright Am. Chem. Soc.

bond formation. This structure shows that the A10 Lys (Lys609) residue is away from the active site and exposed to the solvent, thus Lys609 is not involved in the catalysis of the second half-reaction (fig. 4). A closeup of the active site of the *S. enterica* Acs enzyme (fig. 4B) illustrates the extensive set of interactions of the enzyme with CoA and the alkyl-AMP inhibitor. Detailed structure/function work is now needed to test the strong predictions of this model.

Regulation of *acs* gene expression

In the following sections we discuss what is known about control of the expression of *acs* genes in prokaryotes and eukaryotes. In prokaryotes and eukaryotes *acs* expression is a function of carbon flux, is linked to fatty acid metabolism, and requires histone and nucleoid proteins. Transcriptional regulation of *acs* is best understood in the Gram-negative bacterium *E. coli*. In this bacterium, *acs*

expression is triggered at mid-exponential phase, reaching a maximum as cells enter stationary phase where it gradually decreases as the culture ages [52]. *acs* transcription is repressed by glucose because it is activated by the Crp (catabolite repression protein) protein in response to rising cyclic AMP (cAMP) levels. In this bacterium, *acs* is the promoter-proximal gene of a three-gene operon that includes an acetate transporter (encoded by *actP* [formerly *yjcG*]) and a gene of unknown function, *yjcH* [53]. The ActP function may only be relevant when the concentration of acetate is extremely low in the environment (in the micromolar range), where the gradient is not large enough for free diffusion of this weak acid across the membrane to occur. *acs* is divergently transcribed from *nrfA*, which encodes the periplasmic cytochrome *c552* subunit of formate-dependent nitrite reductase. The intergenic region between *acs* and *nrfA* (280 bp) is a complex regulatory region. Two promoters, one distal (*acsP1*) and one proximal (*acsP2*) control the expression of *acs*, with *acsP2* serving as the major promoter activated by Crp. Activation of *acs* by Crp proceeds via a synergistic class III mechanism, where Crp focuses the housekeeping σ^{70} -RNA polymerase on *acsP2* to generate a productive open complex [54]. The *acs* regulatory region contains two Crp-binding sites, a proximal site (CrpI, centered at -69.5) and a distal one (CrpII, centered at -122.5) [55]. The high-affinity CrpI site is absolutely required for *acs* expression, but optimal expression requires synergistic activation from both sites, i.e. *acsP2* is a Crp-dependent class III promoter (for a comprehensive review of Crp-dependent activation see [56]). Three binding sites for each, the Fis (factor for inversion stimulation; FisI-III) and Ihf (integration host factor; IhfI-III) proteins, are also present in the *acs* regulatory region. Fis and Ihf are nucleoid proteins with a histone-like fold that help organize bacterial chromatin. The FisII (centered at -98) and FisIII (centered at -59) sites are in close proximity to or overlap with the Crp sites [57]. Results from in vitro transcription experiments show that these nucleoid proteins serve as anti-activators of Crp [57]. Fis and Ihf independently reduce *acs* expression. The regulatory interplay controlling *acs* expression is best understood with the model proposed by Browning et al., which is based on the variations in the levels of Crp, Ihf and Fis during the life of a culture [57]. The following facts serve as backdrop for the model. It is known that Fis levels are highest upon dilution into fresh medium and undetectable by stationary phase [58], and that Ihf levels steadily increase, becoming highest in stationary phase [59]. In the Browning et al. model, the low levels of *acs* expression in young cultures are due to high levels of Fis and the absence of Crp. At the point of maximal *acs* expression, Fis levels are undetectable, Ihf levels are on the rise and Crp levels are highest. While Crp levels do not decrease in stationary phase, the levels of Ihf continue to increase.

High levels of Ihf are likely responsible for the decrease in *acs* expression because it can simultaneously bind with Crp to the *acs* regulatory region and block Crp-dependent activation of *acsP2* expression.

Indirect effects also affect *acs* expression. Because Fis represses *crp* expression [60], *acs* expression is low when Fis levels are high. This effect by Fis is overcome by the repression of *fis* by Fis, and once Fis levels are lowered, *crp* expression increases; hence *acs* expression increases as well. The repressive effect of Fis on *acs* expression is further reduced by the negative effect of Crp on *fis* expression [61].

Other indirect effects are not well understood. The glyoxylate bypass repressor IclR and its activator FadR, and the levels of acetate-catabolizing enzymes, affect *acs* expression [52, 62]. These effects are likely indirect, yet important because they probably reflect links between Ac-CoA levels and fatty acid biosynthesis, a major consumer of Ac-CoA. The observed secondary role of transcription factor σ^S (encoded by *rpoS*) in *acs* expression [62] may be related to the fact that σ^S function is needed to respond to the intracellular acidification caused by the release of a proton upon entry of short-chain fatty acids such as acetate and propionate [63]. The existence of a mechanism that transduces a signal caused by the acidification of the cytosol into the *acs* transcription activation system remains a possibility.

The regulation of *acs* transcription in Gram-positive bacteria is not well understood. In *Bacillus subtilis* the carbon flux regulator CcpA (for a review on CcpA function see [64]) is required for *acs* transcription activation [65]. The involvement of CcpA in *acs* expression is consistent with regulatory strategies observed in Gram-negative bacteria.

Expression of *acs* genes in eukaryotes

Eukaryotes typically have two isoforms of Acs, one in the cytosol, the other one in the mitochondrion [23–29]. The different locations of Acs reflect the fate of Ac-CoA. Activation of acetate to Ac-CoA by Acs occurs in the cytosol where Ac-CoA is consumed by fatty acid biosynthesis and many other anabolic processes [30], whilst Ac-CoA-dependent energy generation occurs in the mitochondrion. To catabolize cytosolic Ac-CoA, the acetyl moiety is transferred to L-carnitine by the carnitine acetyltransferase enzyme, brought into the mitochondrion, transferred back to CoA, then used to generate energy via the tricarboxylic acid cycle or to generate important metabolites via the glyoxylate bypass [31, 32]. In *Saccharomyces cerevisiae*, ACS1 is a mitochondrial enzyme needed for growth on acetate under gluconeogenic conditions, and ACS2 is a cytosolic enzyme that supplies the cell with the Ac-CoA required for anabolic processes during anaero-

bic growth on glucose [25, 66]. The ACS isoenzymes are central to yeast metabolism. While the disruption of *ACS1* causes an extended lag phase in cultures growing aerobically on glucose, ethanol or acetate, a strain lacking *ACS2* cannot grow on glucose. The observation that an *ACS1 ACS2* double mutant of *S. cerevisiae* is not viable suggests other sources of Ac-CoA (e.g. pyruvate) cannot compensate for the lack of ACS [25, 26].

As in bacteria, the transcriptional regulation of yeast *ACS1* gene expression is complex since it involves positive and negative effectors [67]. The recurrent themes are the involvement of regulators that monitor carbon flux, and links to the regulation of genes involved in fatty acid metabolism.

In *S. cerevisiae*, *ACS1* expression is severely repressed by high concentrations of glucose or other fermentable carbon and energy sources in the culture medium. Under conditions of growth on non-fermentable carbon sources (e.g. ethanol, acetate) or sugar limitation, *ACS1* expression increases >2 orders of magnitude over the repressed levels. Positive control of *ACS1* transcription is mediated by a carbon source-responsive element (CSRE) similar to the one that controls the expression of structural genes of the glyoxylate bypass [68, 69], and by the transcriptional activator Adr1p [70]. The CSRE directs the Cat8p transcription activator of glucose-repressible genes [71–73]. Negative control of *ACS1* transcription is exerted by the Ume6p repressor protein [74], which binds to URS1 motifs present in the *ACS1* promoter [75].

The regulatory region of *ACS2* contains a consensus inositol/choline-responsive element (ICRE) that serves as binding site to the heterodimeric Ino2p/Ino4p activator complex [76], and three binding sites for transcription factor Abf1p, a known activator of ribosomal proteins, glycolytic enzymes and fatty acid biosynthetic genes [77–80]. These results reflect the integration of expression of *ACS2* and fatty acid biosynthesis.

S. cerevisiae and the Crabtree (inhibition of respiration by glycolysis)-negative yeast *Kluyveromyces lactis* share many of the genes encoding functions needed for carbon and energy metabolism, including *ACS1* and *ACS2*. Unlike in *S. cerevisiae*, *K. lactis* growth on glucose does not completely repress *ACS1* expression, and Cat8p controls the expression of both *ACS1* and *ACS2* genes [81]. The reason for this simplification in *ACS* gene regulation is unclear. Similarly to yeast, *ACS* transcriptional control in the fungi *Aspergillus nidulans* and *Aspergillus niger* is controlled by the carbon-flux-regulator FacB protein, a Cat8 ortholog [82, 83].

Mammalian cells also have two ACS isoenzymes. In the liver, cytosolic ACS1 activity rises in response to acetate generated by bacterial metabolism in the large intestine, by histone deacetylases, by the oxidation of ingested ethanol and by the activity of the ubiquitous acetyl-CoA

hydrolase [84–87]. In addition, ACS1 activity rises with insulin in response to a carbohydrate-rich diet, which increases fatty acid synthesis [88, 89]. *ACS1* expression is induced when cells are deprived of sterols, and as expected, sterol regulatory element-binding proteins (SREBPs) mediate the effect of sterols on gene expression [28, 90–92].

In mice, ACS1 messenger mRNA (mRNA) is most abundant in kidneys, ovaries and testes, with lower levels found in liver, brain and heart, and very low but detectable levels in lung and skeletal muscle [27]. The *ACS2* gene in mice encodes a mitochondrial matrix enzyme, and the primary amino acid sequence of its product is 45.8% identical to that of ACS1 [24]. ACS2 mRNA level is highest in heart and skeletal muscle, and absent in the liver. ACS2 levels are induced under ketogenic conditions such as starvation and diabetes, but the mechanisms by which expression is induced remain unclear. Incorporation of radiolabeled acetate into CO₂ and lipids indicates that Ac-CoA produced by ACS2 is mainly used in energy generation [24].

In the plant *Arabidopsis thaliana* the source of acetyl-CoA in the plastid is not known. The question of how acetyl-CoA is generated in plastids is of particular importance because this organelle is responsible for the synthesis of fatty acids. In *A. thaliana*, the level of ACS mRNA during silique (pod) development does not correlate in time and space with lipid accumulation. In contrast, the pattern of accumulation of subunits of plastidic pyruvate dehydrogenase matches that of lipid synthesis in the developing embryo, suggesting that pyruvate dehydrogenase, not ACS, is responsible for the synthesis of Ac-CoA during lipid synthesis for oils in seeds [93]. These findings suggest that pyruvate, not acetate, is transported into plastids for the purpose of generating acetyl-CoA for fatty acid biosynthesis in seeds.

Posttranslational regulation of Acs activity

Knowledge of the posttranslational regulation of Acs activity is recent. Interestingly, the Sir2 (sirtuin)-dependent protein acetylation/deacetylation system (SDPADS) responsible for controlling Acs activity is also involved in the control of gene expression in eukaryotes [94]. From the standpoint of metabolism, probably the most important feature of the SDPADS is that it consumes the valuable cofactor NAD⁺, hence its activity must be tightly controlled by the cell. Acs is the first metabolic enzyme known to be under the control of the SDPADS in prokaryotes [95] and eukaryotes [96]. Activity of the *S. enterica* Acs enzyme is regulated by acetylation of residue Lys609 of the A10 motif (marked with an asterisk in fig. 2), which is essential for the activation of acetate to acetyl-AMP [95]. A single acetylation event completely blocks

acetyl-AMP synthesis, thus blocking growth of *S. enterica* on low concentrations of acetate (≤ 10 mM) [97]. In *S. enterica*, the Acs enzyme is acetylated by a specific, acetyl-CoA-consuming protein acetyltransferase [V. S. Starai and J. Escalante-Semerena, unpublished results]. In this bacterium, acetylated, inactive Acs is reactivated by the NAD⁺-dependent CobB sirtuin protein deacetylase [95, 98]. Removal of the acetyl moiety from Acs consumes one mole of NAD⁺ per mole of acetyl moiety removed, yielding 2'-acetyl-O-ADP-ribose and nicotinamide as products of the reaction [99, 100]. Although the physiological conditions and signals that modulate the acetylation/deacetylation state of Acs are presently unknown, the fact that nicotinamide is a potent inhibitor of the deacetylase enzyme suggests a link between Acs activity (carbon metabolism) and NAD⁺ biosynthesis (energy generation) [101–104]. One could speculate that low NAD⁺ levels may signal a low energy charge to the cell, leading to downregulation of energy-consuming reactions such as the Acs reaction.

At present, the list of protein substrates for the SDPADS is short, and our understanding of how the acetylase and deacetylase enzymes interact with their substrates is limited. Acs should serve as an excellent model system to learn more about these interactions since this enzyme is kinetically well characterized and the conformations that catalyzed both half-reactions are known.

Impact of acetate metabolism on cell lifespan

The fact that Acs activity is posttranslationally controlled by the SDPADS highlights the central role of acetate metabolism in cell physiology. In eukaryotic cells, gene expression is controlled, among other means, by the acetylation state of histones, with hypoacetylated histones leading to gene silencing [105]. Under calorie-restricted conditions, *S. cerevisiae* cells silence gene expression with hypoacetylated histones and divert carbon metabolism into the mitochondrial Krebs cycle to increase the respiration rate. Under these conditions cell lifespan is extended [106]. Acetate generated from the sirtuin-dependent deacetylation of histones is likely to be reactivated to Ac-CoA by Acs and reenter central metabolism.

Concluding remarks

The wealth of information accumulating in genome databases will continue to facilitate the study of genes encoding acetyl-CoA synthetases. In the foreseeable future structure/function studies of Acs enzymes will provide details of the mechanism of product formation, and will dissect the contributions of specific residues to catalysis and structure. Future work on the regulation of *acs* gene

expression will unveil details of the complex interplay amongst regulatory systems and will identify metabolic signals that feed into these systems to precisely control *acs* gene expression, ultimately expanding our understanding of metabolic integration. Acs will serve as a model system to further our understanding of the post-translational machinery controlling its activity, and probably those of many other important enzymes. The stage is set.

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