The Two Acetyl-coenzyme A Synthetases of Saccharomyces cerevisiae Differ with Respect to Kinetic Properties and Transcriptional Regulation*  

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Saccharomyces cerevisiae contains two structural genes, ACS1 and ACS2, each encoding an active acetyl-coenzyme A synthetase. Characterization of enzyme activities in cell-free extracts from strains expressing either of the two genes revealed differences in the catalytic properties of the two enzymes. The $K_m$ for acetate of Acs1 was about 30-fold lower than that of Acs2, but Acs2 could use propionate as a substrate. Enzyme activity measurements and mRNA analyses showed that ACS1 and ACS2 were both expressed during carbon-limited growth on glucose, ethanol, and acetate in aerobic chemostat cultures. In anaerobic glucose-limited cultures, only the ACS2 gene was expressed. Based on these facts, the products of the ACS1 and ACS2 genes were identified as the previously described “aerobic” and “non-aerobic” forms of acetyl-coenzyme A synthetase, respectively. Batch and glucose-pulse experiments revealed that transcription of ACS1 is subject to glucose repression. A mutant strain lacking Acs2 was unable to grow on glucose in batch cultures, but grew readily in aerobic glucose-limited chemostat cultures, in which the low residual glucose concentration alleviated glucose repression. Experiments in which ethanol was pulsed to aerobic ethanol-limited chemostat cultures indicated that, in addition to glucose, ethanol also repressed ACS1 transcription, although to a lesser extent. In contrast, transcription of ACS2 was slightly induced by ethanol and glucose. Absence of ACS2 prevented complete glucose repression of ACS1, indicating that ACS2 (in)directly is involved in the transcriptional regulation of ACS1.

When Saccharomyces cerevisiae grows on acetate or ethanol, ATP-dependent activation of acetate to acetyl-coenzyme A is catalyzed by acetyl-coenzyme A synthetase (EC 6.2.1.1). In addition to serving as the fuel for the citric acid cycle, acetyl-coenzyme A is an essential building block for the synthesis of lipids and some amino acids. During growth on glucose, direct formation of acetyl-coenzyme A from pyruvate is catalyzed by the mitochondrial pyruvate dehydrogenase complex. Alternatively, conversion of pyruvate into acetyl-coenzyme A can be accomplished by the concerted action of the enzymes of the pyruvate dehydrogenase bypass: pyruvate decarboxylase, acetaldehyde dehydrogenase, and acetyl-coenzyme A synthetase (1, 2). Recent work has indicated that the latter pathway is essential for growth, probably for the provision of cytosolic acetyl-coenzyme A required for lipid synthesis (3, 4).

In S. cerevisiae, a completely respiratory sugar metabolism is only observed at relatively low growth rates in aerobic, sugar-limited cultures (e.g. chemostat cultures). Upon exposure of such cultures to high sugar concentrations, metabolism becomes respirofermentative and pyruvate metabolism occurs predominantly via pyruvate decarboxylase (5, 6). Under such conditions acetate is formed as a by-product, indicating that the in vivo activity of acetaldehyde dehydrogenase exceeds that of acetyl-coenzyme A synthetase. Acetate production can be a major problem in industrial high-biomass-density cultures of S. cerevisiae (e.g. for heterologous protein production), because this weak organic acid dissipates the pH gradient across the plasma membrane, leading to a reduction of the biomass yield (7).

In view of the central role of acetyl-coenzyme A synthetase in the carbon metabolism of S. cerevisiae, it is not surprising that the biochemistry of this enzyme has been studied in detail. Two immunologically distinct forms of the enzyme have been described (8). The two forms differed with respect to kinetic properties, substrate specificity, and cellular localization (9–11) and, based on their levels in shake-flask cultures, were called “aerobic” and “non-aerobic” acetyl-coenzyme A synthetase (12). The differences in regulation, localization, and kinetic aspects suggest different roles in metabolism for both enzymes. Only the aerobic form of the enzyme has been purified to homogeneity (13, 14), and it has remained unclear whether the two forms are encoded by different genes.

Recently two structural genes, ACS1 (15) and ACS2 (3), each encoding acetyl-coenzyme A synthetase, have been cloned from S. cerevisiae. Disruption of both genes was lethal (3), indicating that acetyl-coenzyme A synthetase is an essential enzyme in S. cerevisiae. Strains in which only ACS2 was disrupted grew normally on ethanol or acetate, but were unable to grow on glucose in batch cultures (3). This may be related to the observation that ACS1 is subject to glucose repression (16). In contrast, acs1 mutants grow well on glucose (3).

The aim of the present study was to investigate the physiological function and regulation of both ACS proteins, and to determine whether they correspond to the aerobic and non-aerobic forms of acetyl-coenzyme A synthetase. To this end, kinetic properties of acetyl-coenzyme A synthetase were compared in cell-free extracts of isogenic wild type, acs1 and acs2 S. cerevisiae strains. Furthermore, the transcriptional regulation of both genes was studied in batch and chemostat cultures.
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MATERIALS AND METHODS

Yeast Strains—Yeast strains used for detailed physiological studies should preferably lack auxotrophic markers, and, whenever mutants are used, these should be isogenic to the wild type. These criteria were met by using the set of isogenic strains constructed in a previous study (3): S. cerevisiae Tz2-3D (HO/HO ACS1/ACS1 ACS2/ACS2), G6621 (HO/HO acs1::APT1/acs1::APT1 ACS2/ACS2), and G6625 (HO/HO ACS1/acs2::Tn5ble/acs2::Tn5ble).

Growth Conditions and Media—For small-scale pilot experiments, cells were grown at 30°C in 750-ml shake-flasks (250 rpm) containing 100 ml of mineral medium (17) supplemented with glucose (10 gl−1), ethanol (30 gl−1), or acetate (5 gl−1).

Chemostat cultivation was performed in 2-liter laboratory fermenters (Applikon, Schiedam, The Netherlands), at a dilution rate of 0.10 h−1. The working volume of the culture was kept at 1.0 liter via an electrical level sensor. Removal of effluent from the center of the culture ensured that biomass concentrations in the effluent line differed by less than 1% from those in the culture. The temperature was kept at 30°C, and the pH was maintained at 5.0 by automatic addition of 2.0 M KOH.

To maintain aerobic conditions, an air flow of 0.3 liter per min through the culture was maintained using a Brooks S876 mass-flow controller and the culture was stirred at 800 rpm. The dissolved oxygen concentration, continuously measured with an Ingold polarographic oxygen electrode, remained above 25% of air saturation in all aerobic experiments. The temperature was kept at 30°C, and the medium was sparged with nitrogen. To minimize oxygen diffusion, anaerobic cultures were equipped with Norpren tubing. Chemostat data refer to steady state cultures without detectable metabolic oscillations. Culture purity was evaluated by phase-contrast microscopy at 1000× magnification and plating on selective media.

The mineral medium used in the chemostat experiments has been described (18). Carbon sources were added at a concentration of: 10 gl−1 glucose, 7.5 gl−1 ethanol, or 10 gl−1 acetate. For anaerobic cultivation, the medium was supplemented with the anaerobic growth factors Tween 80 and ergosterol as described (19) and the glucose concentration was 25 gl−1.

Pulse Experiments—After glucose-limited steady state cultures had been obtained, glucose was added by means of a syringe to give an initial concentration of 75 mm. Ethanol (40 mm) and combined ethanol-acetic acid (20 mm each) pulses were added to ethanol-limited steady state cultures. Throughout the pulse experiments, the medium flow into the fermenter and the removal of effluent were continued. The effluent was cooled on ice immediately after leaving the fermenter. At appropriate intervals, samples were collected from the effluent line and used for metabolite determination and mRNA analysis.

Determination of Dry Weight—Dry weights of culture samples (10 ml) were determined using nitrocellulose filters (pore size 0.45 µm; Gelman Sciences). After removal of the medium by filtration, the filters were washed with demineralized water and dried in a Sharp R-7400 microwave oven for 20 min. Parallel samples varied by less than 1%.

Substrate and Metabolite Analysis—Glucose was determined with the Merck glucose-oxidase kit (catalog no. 14143). Ethanol was assayed with a colorimetric assay kit (EK 003, Leeds Biochemicals Ltd., Leeds, United Kingdom). Acetate was assayed using the Boehringer Mannheim acetic acid kit (catalog no. 148261).

Preparation of Cell-free Extracts—Cells (approximately 100 mg dry weight) were harvested by centrifugation at 5,000 × g for 10 min, washed once with 100 mM potassium-phosphate buffer (pH 7.5, 4°C), and resuspended in 100 mM potassium-phosphate buffer pH 7.5, 2 mM MgCl2, and 1 mM dithiothreitol. Cells were disrupted immediately by sonication with 0.7-mm diameter glass-balls at 0°C for 4 × 30 s using an MSE sonicator (150-watt output, 8 µm peak-to-peak amplitude). Whole cells and debris were removed by centrifugation at 20,000 × g (10 min at 4°C). The clear supernatant, typically containing 2–4 mg of protein/mL−1, was used as cell-free extract. Protein concentrations were measured by the Lowry method.

Enzyme Assays—The specific activity of acetyl-coenzyme A synthetase was determined at 30°C in a Hitachi spectrophotometer at 340 nm. The standard reaction mixture (1 ml) contained 100 µmol of Tris-HCl (pH 7.7), 10 µmol of l-malate (pH 7.7), 0.2 µmol of coenzyme A, 8 µmol of ATP (pH 7.7, 1 µmol of MgCl2), 3 units of malate dehydrogenase, 0.4 unit of citrate synthase, and cell-free extract. The reaction was started with 100 µmol of potassium acetate. Enzyme activities were calculated assuming an extinction coefficient of 6.3 ml cm−1 µmol−1. One unit was defined as the amount of enzyme catalyzing the acetate-dependent formation of 1 µmol of NADH min−1 in the coupled assay. For determination of the Km, the acetyl or ATP concentration in the assay was varied, leaving the other components constant. Kinetic parameters were estimated by nonlinear regression, assuming Michaelis-Menten kinetics, using the program Fig.P (Fig.P Software).

This assay would also measure the combined activity of acetate kinase and phosphotransacetylase, acting together in hydrolyzing acetyl-coenzyme A to generate ATP during anaerobic growth in bacteria (20). However, they can also perform the reverse reaction, which generates acetyl-coenzyme A. To rule out the possibility that this two-step reaction is present in yeast (e.g. either ACS1 or ACS2 encodes an acetate kinase), we tested cell-free extracts from wild type and mutant strains for the presence of phosphotransacetylase by omitting ATP from the reaction mixture and starting the reaction with 10 µmol of acetyl phosphate. Escherichia coli extracts were used as a positive control. No activity was observed in any of the three S. cerevisiae strains (data not shown), indicating that both ACS1 and ACS2 encode genuine acetyl-coenzyme A synthetases.

Activity of acetyl-coenzyme A synthetase with propionate as a substrate was determined in a discontinuous assay, measuring the propionate-dependent consumption of coenzyme A min−1.

Estimation of the Capacities of Acs1p and Acs2p in Wild Type Cultures—Kinetic analysis of acetate activation by cell-free extracts indicated that the acetyl-coenzyme A synthetases encoded by the ACS1 and ACS2 genes both obey Michaelis-Menten kinetics, but exhibited a substrate-dependent difference in Vmax, and Km. The ratio of the two enzymes (Vmax,ACS1/Vmax,ACS2) was estimated by performing enzyme activity assays at two different acetate concentrations (1 and 100 mM, respectively). The ratio of the capacities was calculated from Equation 1:

\[
\frac{V_{\text{max,ACS1}}}{V_{\text{max,ACS2}}} = \frac{K_{\text{m,ACS1}}}{K_{\text{m,ACS2}}} + \frac{1}{100}
\]

In Equation 1, r81 and r100 are the reaction rates observed with 1 and 100 mM acetate, respectively.

RNA Techniques—Total RNA was isolated from 2-ml samples as described (22), except that the final samples were stored in pure formamide. This reduced the total sample volume by a factor of 2, thus avoiding the need to concentrate samples with a low amount of RNA. For Northern blot analysis, approximately 5 µg of each sample were mixed with an equal volume of sample buffer (2 × MOPS), 2.88% formaldehyde, and 0.1 mg/ml ethidium bromide and separated on a 0.7% agarose gel containing 1.44% formaldehyde. The RNA molecules were transferred to a nylon membrane (Boehringer Mannheim) by vacuum blotting in 20 × SSC (3.0 M NaCl, 0.3 M sodium citrate). Hybridization was carried out at 65°C for 18 h in 5 × SSC, 1% SDS, 20 mM sodium pyrophosphate, 0.1% sodium laurylsarcosine, and 1% blocking reagent (Boehringer Mannheim). Probes were prepared as described previously (3). The blots were washed twice for 10 min in 5 × SSC, 1% SDS at 65°C. Quantification of the signals was done using the PhosphorImager system (Molecular Dynamics, B&L Systems, Maarssen, The Netherlands). In all experiments, PDA1 mRNA was used as an internal loading standard. All solutions were made RNase free by autoclaving for 40 min at 120°C. Gel trays were washed overnight with a 10% Glorix solution.

RESULTS

Kinetic Properties of the ACS1 and ACS2 Gene Products—To investigate whether the gene products of the ACS genes correspond to the aerobic and non-aerobic forms of acetyl-coenzyme A synthetase (8, 11, 23), the kinetic properties of the enzymes were compared. Ethanol-limited chemostat cultures of wild type Saccharomyces cerevisiae and isogenic acs1 and acs2 mutants, grown at a dilution rate of 0.10 h−1, were used as a reproducible source of biomass.

The aerobic and non-aerobic forms of S. cerevisiae acetyl-coenzyme A synthetase have been reported to exhibit different affinities for acetate and ATP, with the aerobic form exhibiting

1 The abbreviation used is: MOPS, 4-morpholinepropanesulfonic acid.
lower $K_m$ values for both substrates (9). The $K_m$ for acetate of the strain containing only ACS1 was about 30-fold lower than that of the strain expressing only ACS2 (Table I). In extracts from wild type, the $K_m$ for acetate was close to that of GG625 extracts (Table I). Extracts prepared from the three strains did not exhibit substantial differences with respect to their $K_m$ for ATP (Table I).

A striking difference between the aerobic and non-aerobic forms of acetyl-coenzyme A synthetase is the inability of the latter to activate propionate (9). Extracts from wild type and the isogenic mutant expressing only ACS1 were able to activate propionate, albeit at lower rates than acetate (Table I). In contrast, activation of propionate was not observed with extracts from the strain expressing only ACS2 (Table I). From these data we concluded that the aerobic protein is encoded by the ACS1 gene and non-aerobic protein by the ACS2 gene.

**Growth of an acs2 Mutant in Glucose-limited Chemostat Cultures**—S. cerevisiae mutants in which the ACS2 gene has been disrupted are unable to grow on glucose in batch cultures (3). This may be due to repression of ACS1 by glucose (16). Alternatively, it may indicate that Ac2p has an indispensable function in glucose metabolism that cannot be met by Acs1p (e.g. due to different catalytic properties or subcellular compartmentation).

In a preliminary study, transcriptional regulation of both genes was studied in shake-flask cultures of wild type yeast grown on glucose. ACS1 mRNA was not detectable during exponential growth, but appeared when the culture entered stationary phase (Fig. 1). Apparently, glucose repression of ACS1 was relieved when glucose was consumed and the culture switched to consuming ethanol and acetate produced during exponential growth. In contrast, ACS2 mRNA was observed throughout the growth curve (Fig. 1). Experiments in which glucose was added to acs2-grown batch cultures indicated that repression of ACS1 transcription started at glucose concentrations of 100 mg liter$^{-1}$ (data not shown).

The low residual substrate concentration in glucose-limited chemostat cultures is known to alleviate glucose repression (24, 25). We therefore tested whether a strain expressing only ACS1 was able to grow under these conditions by switching an ethanol-limited chemostat culture to medium with glucose as the sole carbon source. The strain rapidly adapted to glucose-limited growth. In the resulting glucose-limited steady state cultures its biomass yield was the same as that of the wild type strain (Table II). This indicated that glucose repression of ACS1 is the sole reason for the inability of acs2 mutants to grow on glucose in batch cultures.

**Regulation of ACS Genes in Carbon-limited Chemostat Cultures**—To investigate effects of carbon source on the regulation of both genes, their expression was studied in carbon-limited chemostat cultures. In contrast to batch cultivation, chemostat cultivation can be carried out with a constant dissolved-oxygen concentration and growth rate, which is known to have substantial effect on acetyl-coenzyme A synthetase levels in S. cerevisiae (26). This enables studies on the regulation by carbon source without interference.

As the $K_m$ values of the ACS gene products for acetate are different (Table I), the ratio of their maximum activities in cell-free extracts ($V_{\text{max,ACS1}} / V_{\text{max,ACS2}}$) can be estimated by measuring acetyl-coenzyme A synthetase activities at two substrate concentrations (see “Materials and Methods”). These estimations indicated that in aerobic, carbon-limited chemostat cultures grown on glucose, ethanol, or acetate, both genes were expressed simultaneously (Table II). Under these conditions, ACS1 was responsible for most of the total capacity (Table II). In the aerobic cultures grown on ethanol and glucose, expression levels of ACS2 were not substantially different, whereas the expression of ACS1 was higher in ethanol-limited cultures (Table II).

In anaerobic glucose-limited chemostat cultures, ACS activity was lower than in aerobic cultures and appeared to be encoded exclusively by the ACS2 gene (Table II). This is in line with the identification of ACS2 as the gene encoding the non-aerobic enzyme. Nevertheless, it should be noted that ACS2 expression is not limited to anaerobic or fermentative growth conditions (Table II).

Northern analysis corroborated that in aerobic carbon-limited chemostat cultures grown on glucose, ethanol, or acetate,
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Transcription of ACS1 and ACS2 during Transition from Glucose-limited to Glucose-excess Conditions—In the experiments discussed above, growth was studied under carbon-limited conditions. Although industrial fed-batch cultures of S. cerevisiae are in principle sugar-limited, transient exposure to high sugar concentrations is inevitable due to imperfect mixing and the high sugar concentration in the feed of large scale bioreactors. On a laboratory scale, this situation can be simulated by glucose pulses to aerobic, glucose limited cultures. One of the consequences of such transient exposure of aerobic grown S. cerevisiae cultures to excess glucose is the accumulation of ethanol and acetate, the so-called short term Crabtree effect (27). Since the capacity of acetyl-coenzyme A synthetase may be a relevant factor in the occurrence of acetate formation, transcriptional regulation of the ACS genes was studied after addition of 75 mM glucose to aerobic, glucose-limited yeast cultures.

After applying the glucose pulse, three metabolic phases could be discerned (Fig. 2A). During the first phase (0–160 min), the glucose added to the culture was rapidly consumed. Glucose metabolism was accompanied by the production of ethanol and, to a lesser extent, acetate. In the second phase (170–380 min), ethanol was consumed, resulting in the formation of more acetate. This was consumed in the third phase (390–420 min), the glucose added to the culture was rapidly consumed. The levels of ACS1 and ACS2 mRNA during these phases were monitored by Northern analysis (Fig. 2B).

Transcription of ACS1 decreased below the detection limit within 10 min after addition of the glucose (Fig. 2B), confirming ACS1 glucose repression. As soon as glucose was exhausted and ethanol consumption started, ACS1 mRNA reappeared. During this phase ACS1 mRNA levels were 2–4-fold higher, compared to the steady state level (Fig. 2B). As the ethanol concentration in the culture decreased below 10 mM and acetate consumption started, a sharp increase of mRNA occurred (Fig. 2B). The maximum level, observed after 410 min, corresponded to approximately 40-fold the steady state value.

Throughout the pulse experiment, the levels of ACS2 mRNA varied between 60 and 180% of the steady state value, suggesting that transcription of ACS2 is relatively insensitive to the transient accumulation of metabolites. However, ACS2 completely disappeared at the end of the experiment (Fig. 2B). Only after several hours did ACS2 mRNA return to its steady state level (data not shown).

Regulation of ACS1 Expression by Ethanol—It is obvious that full transcription of ACS1 only occurred when ethanol is completely consumed (Fig. 2B). Either the gene is relieved from ethanol repression, or the increased level of acetate might induce ACS1 transcription. To further investigate the regulation by ethanol, C-2 carbon pulses were given to ethanol-limited chemostat cultures. Since, full transcription of ACS1 occurred when the concentrations of ethanol and acetate were approximately 10–20 mM, we first added a mix of 20 mM ethanol and 20 mM acetate to an ethanol-limited culture. If the disappearance of any residual glucose was responsible for the increase of ACS1 mRNA levels (Fig. 2), 350-min sample and greater), no increase of transcription is expected in this new experiment. On the other hand, repression by ethanol would lower the levels of ACS1 mRNA, whereas induction by acetate

### Table II

| Strain  | Relevant genotype | Carbon source | Acs activity | \( \frac{V_{\text{max,ACS1}}}{V_{\text{max,ACS2}}} \) | Biomass yield |
|---------|------------------|---------------|--------------|---------------------------------|---------------|
| T2–3D   | ACS1 ACS2        | Glucose       | 0.42         | 4.2                             | 0.5           |
| GG621   | acs1 ACS2        | Glucose       | 0.24         | 0.5                             | 0.5           |
| GG625   | ACS1 acs2       | Glucose       | 0.38         | 0.5                             | 0.5           |
| T2–3D   | ACS1 ACS2        | Ethanol       | 1.26         | 26                              | 0.6           |
| GG621   | acs1 ACS2        | Ethanol       | 0.30         | 0.6                             | 0.6           |
| GG625   | ACS1 acs2       | Ethanol       | 1.04         | 0.6                             | 0.6           |
| T2–3D   | ACS1 ACS2        | Glucose (anaerobic) | 0.08         | <0.01                           | 0.1           |

### Table III

| mRNA | Glucose | Ethanol | Acetate | Glucose (anaerobic) |
|------|---------|---------|---------|---------------------|
| ACS1 | 1       | 1.4     | 1.8     | 0                   |
| ACS2 | 1       | 1.6     | 3.2     | 2.8                 |

**TABLE II**

Acetyl-coenzyme A activities and biomass yields in cell-free extracts from carbon-limited chemostat cultures (D = 0.10 h⁻¹) of wild type S. cerevisiae and mutants in which either ACS1 or ACS2 have been inactivated

Unless otherwise indicated, cultures were grown aerobically. The ratio of the maximum specific activities of the ACS1 and ACS2 gene products was calculated from enzyme activity assays at two different acetate concentrations (see "Materials and Methods").

**TABLE III**

Levels of ACS1 and ACS2 mRNA in steady state carbon-limited chemostat cultures (D = 0.10 h⁻¹) of wild type S. cerevisiae T2–3D

The cultures were aerobic, unless stated otherwise. The levels of the two mRNAs have been normalized to the level of PDA1 mRNA. The level of each transcript during aerobic, glucose-limited growth was set at 1.

![Fig. 2. Transient responses of wild type S. cerevisiae to a glucose pulse.](image-url)

At time zero, 75 mmol of glucose was added to an aerobic, glucose-limited chemostat culture (D = 0.10 h⁻¹) of S. cerevisiae T2–3D. A, concentrations of glucose, ethanol, and acetate. B, relative abundances of ACS1 (●) and ACS2 (○) mRNA. PDA1 mRNA was used as a loading standard; the steady state levels of both transcripts (t = 0) were set at 100%. Note that the y axis is exponential; points drawn on the x axis were below the detection limit.
would increase it. As can be seen from Fig. 3A the level of ACS1 mRNA decreased rapidly, although at a lower rate than after a glucose pulse (Fig. 2B), to approximately 20% of the steady state level. As soon as ethanol and acetate were consumed, ACS1 mRNA returned to the original level. The ACS2 mRNA level, on the other hand, increased to almost 200% before returning to the steady state level at the end of the pulse. These data strongly suggest that ACS1 transcription is repressed by ethanol. This was confirmed in another pulse experiment, in which 40 mM ethanol was added to an ethanol-limited chemostat culture. Both genes responded exactly the same as during the ethanol/acetate pulse.

Apparently, ACS1 is repressed by ethanol and ACS2 is slightly induced by ethanol.

Transgene Regulation of ACS1 Transcription—Since ACS1 and ACS2 catalyze the same reaction, it does not seem unlikely that interactions exist between the regulatory mechanisms affecting the transcription of both genes.

To test whether the transcription of ACS1 is influenced by the presence of an active ACS2 gene, glucose repression of ACS1 expression was compared in wild type and GG625, which lacks an active ACS2 gene. When exponential-phase shake-flask cultures growing on acetate were pulsed with 1 g liter\(^{-1}\) glucose, ACS1 expression in the wild type showed exactly the same pattern as in the chemostat experiment shown in Fig. 2B. Within 15 min after the pulse, ACS1 mRNA could no longer be detected (Fig. 4A). After 60 min the mRNA reappeared. In the acs2 mutant GG625, a decrease of ACS1 transcription was also observed. However, in this case significant levels of ACS1 mRNA remained present throughout the experiment (Fig. 4B). These results, showing that the regulation of ACS1 is influenced by the presence of an active ACS2 gene, were confirmed in glucose-pulse experiments with glucose-limited chemostat cultures of the acs2 mutant (data not shown).

In similar experiments, levels of the ACS2 transcript were monitored after glucose pulses to an acs1 mutant and the isogenic wild type. ACS2 transcription did not differ in the two strains, neither in batch cultures nor in chemostat cultures (data not shown).

**DISCUSSION**

One of the goals of the present study was to determine whether the gene products of the ACS1 and ACS2 genes could be correlated with the aerobic and non-aerobic forms of acetyl-coenzyme A synthetase described previously (8–14). Based on the difference in \( K_m \) for acetate, substrate specificity, and expression under anaerobic conditions, we concluded that ACS1 encodes the aerobic form of the enzyme, whereas ACS2 encodes the non-aerobic form. Identity of the ACS1 gene product and the aerobic isoenzyme is further supported by the correlation

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**Fig. 3. Regulation of ACS1 and ACS2 expression by ethanol.** A, a mix of 20 mmol of ethanol and 20 mmol of acetate was added to an ethanol-limited chemostat culture. Concentrations of ethanol and acetate are shown in the upper panel. The middle panel shows the Northern blot hybridizations, with ACS1/PDA1 and ACS2/PDA1 mRNA, respectively. The relative abundance of ACS mRNAs are depicted in the lower panel.

B, a 40-mmol ethanol pulse was given to an ethanol-limited chemostat culture. Concentrations of ethanol and acetate are shown in the upper panel. The middle panel shows the Northern blot hybridizations, with ACS1/PDA1 and ACS2/PDA1 mRNA, respectively. The relative abundances of ACS mRNAs are depicted in the lower panel.
between the amino acid composition of purified aerobic acetylcoenzyme A synthetase (13) and the predicted protein sequence of the ACS1 gene product (15). So far, the ACS2 gene product (the non-aerobic form) has not been purified to homogeneity. Attempts in our laboratory to achieve this goal were hindered by instability of partially purified preparations of the enzyme.2 We propose that the terms aerobic and non-aerobic acetylcoenzyme A synthetase should no longer be used, because ACS2 is expressed not only during anaerobic growth, but also during aerobic growth on glucose, ethanol, and acetate.

Palmitoyl-coenzyme A was reported to be a strong inhibitor of ACS1-encoded isoform (10). In the present study, palmitoylcoenzyme A was found to be an equally effective inhibitor of acetyl-coenzyme A synthetase activity in cell-free extracts of all three strains (50% inhibition at -25 μM palmitoyl-coenzyme A; data not shown). In contrast to earlier reports (9), no difference was found in K_m for ATP of both gene products. However, it should be taken into account that both the cultivation conditions and assay procedures employed in the previous work (9, 10) were different from those used in the present study.

Consistent with earlier reports (3, 16), ACS1 was found to be subject to glucose repression. Upon exposure of wild type cells to glucose, the level of the ACS1 transcript decreased very rapidly; within 10–20 min after exposure to glucose concentration during the pulse experiments. The high expression of ACS1 during growth on acetate (Fig. 2) suggests that Acs1p is primarily responsible for acetate activation during gluconeogenic growth. The relatively low K_m for acetate of Acs1p enables it to gain enough energy to sustain growth, even at low acetate concentrations. Acs2p, on the other hand, has a lower affinity for acetate, but is expressed during growth on glucose, when the main energy generating flux does not require ACS activity. Therefore, Acs2p is likely to be the major producer of cytosolic acetyl-coenzyme A, in total approximately 4% of the total amount of carbon (4), required for lipid and amino acid biosynthesis.

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