Molecular biology of acetyl-CoA metabolism

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Abstract
We have characterized the expression of potential acetyl-CoA-generating genes (acetyl-CoA synthetase, pyruvate decarboxylase, acetaldehyde dehydrogenase, plastidic pyruvate dehydrogenase complex and ATP-citrate lyase), and compared these with the expression of acetyl-CoA-metabolizing genes (heteromeric and homomeric acetyl-CoA carboxylase). These comparisons have led to the development of testable hypotheses as to how distinct pools of acetyl-CoA are generated and metabolized. These hypotheses are being tested by combined biochemical, genetic and molecular biological experiments, which is providing insights into how acetyl-CoA metabolism is regulated.

Introduction
Acetyl-CoA and malonyl-CoA are the two precursors of de novo fatty acid biosynthesis (Figure 1). The generation of these two metabolites within plastids commits carbon to the biosynthesis of fatty acids. Although the generation of malonyl-CoA, via the carboxylation of acetyl-CoA by acetyl-CoA carboxylase (ACC) is well characterized, how acetyl-CoA is generated within plastids is still a mystery. We are pursuing a combined biochemical and genetic characterization of the roles of plastidic pyruvate dehydrogenase (ptPDH), ATP-citrate lyase (ACL), pyruvate decarboxylase (PDC), aldehyde dehydrogenase (ALDH) and acetyl-CoA synthetase (ACS) in generating the plastidic acetyl-CoA pool. In addition, we are investigating the role of acetyl-CoA metabolism (i.e. acetyl-CoA generation and carboxylation) in regulating de novo fatty acid biosynthesis.

Figure 1
Biochemical mechanisms for generating acetyl-CoA and malonyl-CoA, the precursors of de novo fatty acid biosynthesis

FAS, fatty acid synthase. For other abbreviations, see text.

Key words: fatty acid biosynthesis, malonyl-CoA.

Abbreviations used: ACC, acetyl-CoA carboxylase; ACL, ATP-citrate lyase; ACS, acetyl-CoA synthetase; ALDH, aldehyde dehydrogenase; PDC, pyruvate decarboxylase; ptPDH, plastidic pyruvate dehydrogenase.

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Results and discussion

Genomics of acetyl-CoA metabolism

We have isolated and begun the characterization of Arabidopsis cDNAs and genes coding for the plastidic ACC [1–4], ptPDH [5,6], ACL [7], PDC, ALDH and ACS [5]. These characterizations have identified paralogous genes that code for each subunit of these enzymes. Specifically, the four subunits of the plastidic ACC (biotin carrier subunit, biotin carboxylase and the α and β subunits of the carboxyltransferase) appear to be coded by five genes, four of which are nuclear (CAC1A, CAC1B, CAC2, CAC3) and one plastidic (accD). The ptPDH is composed of four subunits, E1α, E1β, E2 and E3, of which E1α and E2 are each coded by single genes, whereas E1β and E3 are each coded by two genes. The two ACL subunits (ACL-A and ACL-B) are each coded by at least two genes. The single subunit of PDC is coded by two genes (PDC1 and PDC2). ALDH is coded by three genes, two of which (ALDH1 and ALDH3) appear to code for mitochondrial isoenzymes and one (ALDH2) appears to code for a cytosolic isoenzyme. ACS is coded by a single gene.

Acetyl-CoA generation

To ascertain the source of acetyl-CoA required for de novo fatty acid biosynthesis we are conducting two types of experiment: (i) characterization of the expression patterns of the potential acetyl-CoA-producing enzymes (ptPDH, ACL, PDC, ALDH and ACS), which are compared with the expression pattern of ACC; and (ii) characterization of genetic mutants that have altered expression of these enzymes.

The expression patterns of the acetyl-CoA-metabolizing enzymes are being determined by Northern-blot and in situ hybridization studies of the accumulation of each of their respective mRNAs. To date, these studies have revealed that during Arabidopsis seed development the spatial and temporal pattern of ptPDH expression correlates very closely with the expression of the plastidic ACC, but that the expression patterns of ACL and ACS do not [4,5,7]. These findings indicate that, at least in developing seeds, the primary source of acetyl-CoA may in fact be pyruvate (via the action of ptPDH), and that ACS and ACL have a minor role in this process.

To test this hypothesis further, plants that have reduced accumulation of each of these enzymes are being characterized. The accumulation of each of these enzymes is being down-regulated by the expression of antisense RNAs, using the ubiquitously expressing 35 S promoter. Characterization of the antisense-ACL plants, and molecular characterization of the ACL genes and enzyme clearly indicate that this enzyme generates the cytosolic acetyl-CoA pool, and is not directly involved in supplying this precursor for de novo fatty acid biosynthesis.

Down-regulation of ACS does not affect the morphology or growth characteristics of the plant. This contrasts with mutations that block de novo fatty acid biosynthesis (either by a mutation in the enoyl-ACP reductase gene [8], or by antisense down-regulation of ACC). Both of these latter mutations result in plants with reduced growth, and these plants produce leaves that have a variegated yellow phenotype. These differences between the antisense-ACS plants and plants blocked in fatty acid biosynthesis support the hypothesis that ACS is not the primary source of acetyl-CoA for de novo fatty acid biosynthesis. Additional evidence in support of this conclusion was obtained from biochemical characterizations of the antisense-ACS mutant plants. Specifically, the fatty acid content of these plants is unaltered relative to their wild-type siblings. Yet, whereas the rate of in vivo fatty acid biosynthesis from [14C]acetate is reduced in direct proportion to the reduction in ACS accumulation, the rate of in vivo fatty acid biosynthesis from 14CO2 is unaffected by the reduction in ACS accumulation.

In total, these biochemical and genetic characterizations indicate that ACS and ACL are probably not the physiological sources of acetyl-CoA for fatty acid biosynthesis. We are currently undertaking similar characterizations of ptPDH mutants that will ascertain whether this enzyme is the source of the acetyl-CoA pool required for fatty acid biosynthesis.

These findings beg the question as to the physiological role of ACS. As a means of answering this question we are seeking the physiological origin of acetate. Our working hypothesis is that acetate is derived from pyruvate via the sequential action of pyruvate and ALDH, and may be a means of recovering carbon following the anaerobic stress of tissues.

Regulation of fatty acid biosynthesis

To determine whether the generation of precursors (acetyl-CoA and malonyl-CoA) is a significant regulatory locus for controlling fatty acid biosynthesis, we are generating a series of plants that have serially reduced levels of acetyl-CoA-metabolizing enzymes (i.e. ACL, ACS, ptPDH...
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and ACC. To date we have found that reducing the expression of the plastidic ACC by even 20–30% of wild-type levels has a dramatic effect on the growth characteristics of the resulting plants. We suggest that these findings indicate that ACC plays a critical role in the regulation of fatty acid biosynthesis. The transgenic Arabidopsis lines that have reduced ACC expression offer a unique set of reagents for evaluating the effect of limiting membrane biogenesis (by limiting fatty acid biosynthesis) on the growth and development of an organism.

References

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Molecular biology of cytosolic acetyl-CoA generation

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Abstract

ATP citrate lyase (ACL) catalyses the ATP-dependent reaction between citrate and CoA to form oxaloacetate and acetyl-CoA. Our molecular characterizations of the cDNAs and genes coding for the Arabidopsis ACL indicate that the plant enzyme is heteromeric, consisting of two dissimilar subunits. The A subunit is homologous to the N-terminal third of the animal ACL, and the B subunit is homologous to C-terminal two-thirds of the animal ACL. Using both ACL-A- and ACL-B-specific antibodies and activity assays we have shown that ACL is located in the cytosol, and is not detectable in the plastids, mitochondria or peroxisomes. During seed development, ACL-A and ACL-B mRNA accumulation is co-ordinated with the accumulation of the cytosolic homomeric acetyl-CoA carboxylase mRNA. Antisense Arabidopsis plants reduced in ATP citrate lyase activity show a complex phenotype, with miniaturized organs, small cell size, aberrant plastid morphology and reduced cuticular wax. Our results indicate that ACL generates the cytosolic pool of acetyl-CoA, which is the substrate required for the biosynthesis of a variety of phytochemicals, including cuticular waxes and flavonoids.

Introduction

Cast as a central molecule in plant metabolism, acetyl-CoA plays a major role in many interconnecting biochemical pathways. While crucial for de novo fatty acid biosynthesis in plastids and energy production in the mitochondrial tricarboxylic acid cycle, acetyl-CoA is also required for the biosynthesis of secondary phytochemicals essential to plant growth, development and protection, including flavonoids, stilbenoids and isoprenoids, such as sterols and hormones, elongated fatty acids, cysteine, malonyl derivatives and malonic acid [1].

In plants, acetyl-CoA is present in at least four separate compartments: plastids, mitochondria, microbodies and the cytosol [1]. Since acetyl-CoA is membrane-impermeable [2], synthesis is thought to occur in the subcellular compartment where it is required [3,4]. Acetyl-CoA’s compartmentation and plurality of roles in plant metabolism and development complicate the discovery