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Plant one-carbon metabolism and its engineering

Andrew D. Hanson, Douglas A. Gage and Yair Shachar-Hill

The metabolism of one-carbon (C_1) units is vital to plants. It involves unique enzymes and takes place in four subcellular compartments. Plant C_1 biochemistry has remained relatively unexplored, partly because of the low abundance or the lability of many of its enzymes and intermediates. Fortunately, DNA sequence databases now make it easier to characterize known C_1 enzymes and to discover new ones, to identify pathways that might carry high C_1 fluxes, and to use engineering to redirect C_1 fluxes and to understand their control better.

One-carbon (C_1) metabolism is essential to all organisms. In plants, it supplies the C_1 units needed to synthesize proteins, nucleic acids, pantothenate and many methylated molecules[1]. Fluxes through C_1 pathways are particularly high in plants that are rich in methylated compounds such as lignin, alkaloids and betaines because methyl moieties make up several percent of their dry weight[2]. Transfers of C_1 units are also central to the massive photorespiratory fluxes that occur in all C_3 plants[3]. In spite of the fundamental significance of these roles, and the interest in the metabolic engineering of lignin[2], betaines[4] and photorespiration[3], there is much that is not understood about the enzymes, pathways and regulatory mechanisms of plant C_1 metabolism. In part this is because of the obstacles that C_1 metabolism presents for classical biochemistry and genetics: its enzymes can be of low abundance and/or exist as several isoforms, mutants are lacking, and its key intermediates – C_1 substituted folates – are labile and hard to quantify. Fortunately, classical approaches to C_1 metabolism can now be complemented by genomics-driven approaches that exploit the fast-growing DNA sequence databases. Accordingly, this review has three aims:

- To illustrate how genomics-based approaches are advancing our knowledge of plant C_1 biochemistry.
- To bring together biochemical and genomics-derived data to show which C_1 pathways might operate in plants, and where they operate in the cell.
- To examine progress towards engineering C_1 metabolism.

Nucleotide sequence information – from genomes, cDNAs and ESTs – can be used to complement biochemical approaches in several ways. Because most enzymes of C_1 metabolism are highly

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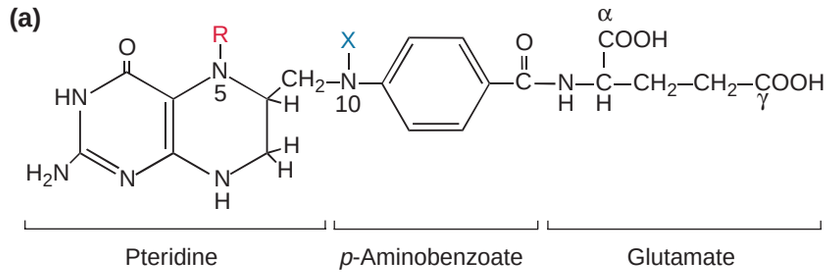
conserved, homology with bacterial, yeast or animal sequences can identify DNA sequences for known plant C_1 enzymes. The proteins that these sequences encode can then be characterized by expression in heterologous systems. Homology searches can suggest the presence (or absence) of enzymes for which there is no biochemical information in plants. The differential expression of genes can be inferred from variation in the count of their cognate ESTs, and this can provide valuable clues about the levels at which pathways might be operating[5]. ESTs, cDNAs and genomic sequences can also give information about the organellar targeting of enzymes via their characteristic signal sequences, and about the sizes of gene families. Lastly, the availability of sequences from a wide range of plants can speed up the preparation of the cDNAs that are needed to make constructs for metabolic engineering.



Folate	R	X
THF	H	H
10-Formyl-THF	H	CHO
5-Formyl-THF	CHO	H
5-Methyl-THF	CH_3	H
5,10-Methenyl-THF	=CH–	
5,10-Methylene-THF	$-CH_2-$	

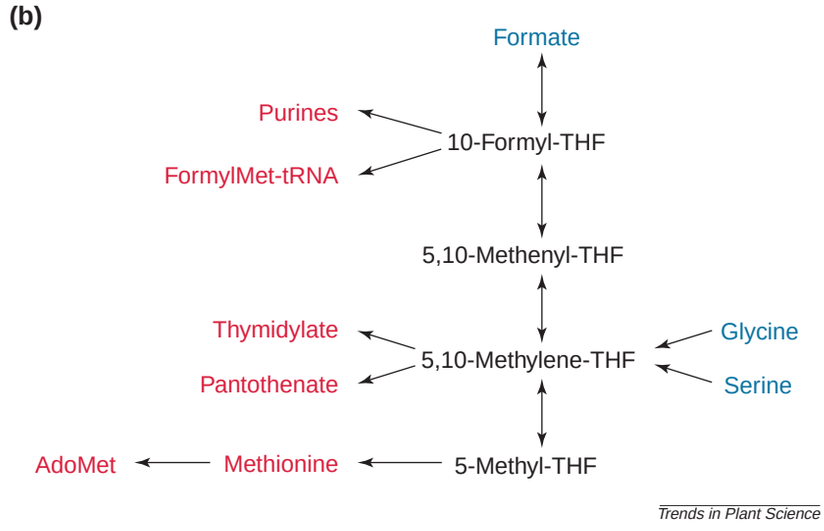


Fig. 1. Tetrahydrofolate (THF) and its role in C_1 interconversions and transfers. (a) Chemical structures of THF and its C_1-substituted derivatives. Plant folates, like those of other organisms, are predominantly present as γ-glutamyl conjugates in which a short γ-linked polyglutamyl chain of up to about eight residues is added to the glutamate moiety[1]. Most folate-dependent enzymes have greater affinity for the polyglutamate forms than for the corresponding monoglutamyl folates[1]. (b) Major C_1 unit interconversions and transfers involving THF derivatives. Sources of C_1 units are shown in blue and their metabolic fates in red.

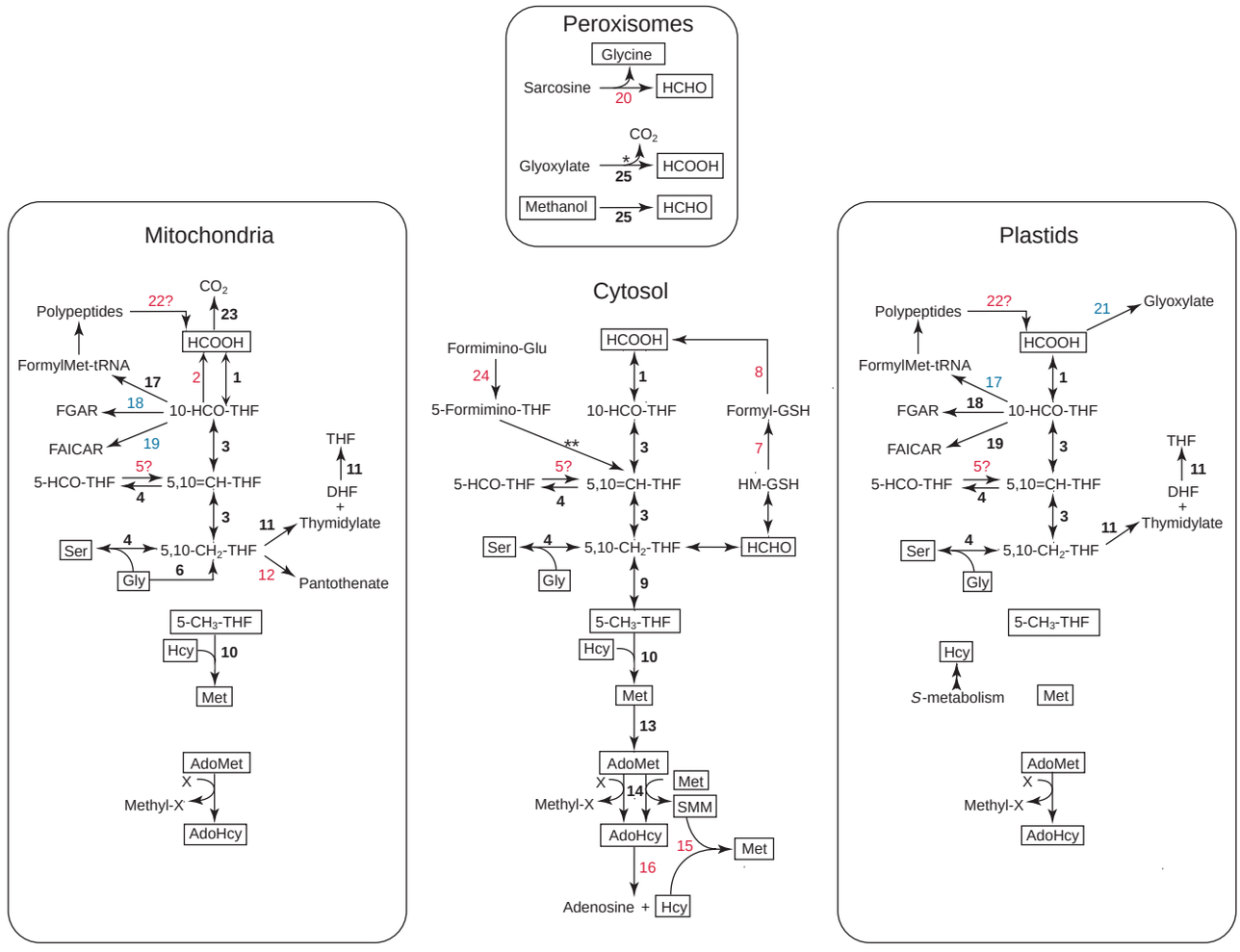
Role of folates in one-carbon metabolism

Here we briefly introduce folate-mediated C_1 metabolism (reviewed in Ref. 1) to provide background for the rest of the article. Many C_1 transfers are mediated by the coenzyme tetrahydrofolate (THF). In essence, catab-olism of serine, glycine and other molecules generates specific C_1 derivatives of THF that are then interconverted between different oxidation states, ranging from 10-formyl-THF (most oxidized) through 5,10-methenyl- and 5,10-methylene- to 5-methyl-THF (most reduced; Fig. 1). These interconversions of C_1-substituted folates form the core of C_1 metabolism, from which C_1 units are withdrawn by anabolic reactions. The largest anabolic flux is the use of 5-methyl-THF to convert homocysteine to methionine, which is incorporated into proteins or converted to *S*-adenosylmethionine (AdoMet), the donor for methylations. In other anabolic reactions, 10-formyl-THF is used to synthesize purines and formylmethionyl-tRNA (for translation initiation in plastids and mitochondria), and 5,10-methylene-THF is used to produce thymidylate and pantothenate (Fig. 1).

Compartmentation of plant one-carbon metabolism

Folate-mediated and other C_1 reactions in plants are highly compartmented (Fig. 2). The overall picture is like that for other eukaryotes[6] inasmuch as many C_1 enzymes occur in both the cytosol and the mitochondria. However, most of them are also found in plastids, and two enzymes are specific to peroxisomes. The data point to some surprising deductions about gaps in pathways and the inter-organellar traffic in C_1-related metabolites that these gaps imply. Although the deductions presented here are robust inasmuch as they are based on concordance between DNA sequence data and biochemical findings, both types of information are subject to caveats. Thus, the algorithms available for predicting proteins from genomic DNA and organellar targeting from the protein sequence are fallible, plant EST collections are far from a complete inventory of the transcriptome, and the *Arabidopsis* genome is only ~80% complete. 'Missing' enzymes, especially organellar ones, might therefore yet be found. Also, clearcut results on enzyme compartmentation can be hard to achieve because of the difficulties in obtaining highly purified subcellular fractions. The following deductions should therefore be viewed as provisional and as stimuli for further investigation.

- The cytosol, mitochondria and plastids all contain the activities required to convert formate to 10-formyl-THF, to interconvert 10-formyl-, 5,10-methenyl- and 5,10-methylene-THF, and to generate 5,10-methylene-THF from serine. However,



Trends in Plant Science

Fig. 2. The reactions of plant one-carbon (C_1) metabolism and their probable compartmentation as deduced from biochemical and DNA sequence evidence. Compartmentation was inferred from sequence data by examining the predicted polypeptides encoded by C_1-related genes, cDNAs and ESTs for well-defined organellar targeting signals. The enzymes corresponding to the numbered reactions are listed in Table 1, together with corresponding literature citations and/or GenBank Accession numbers. The abundance of ESTs for these enzymes in dBEST is given in Figure 4. Different types of evidence were used to assign compartmentation, as indicated by the different colors: bold, black type indicates biochemical and DNA sequence data; red indicates DNA sequence data only; blue indicates biochemical data only. Boxed metabolites are those most likely to be transported (or to diffuse) across organellar membranes. The single asterisk marks the glyoxylate decarboxylation reaction, which is probably mainly non-enzymatic in leaves, but can also be mediated by the peroxidatic action of catalase (enzyme 25) (Refs 18,20). The double asterisk marks the reaction mediated by 5-formimino-THF cyclodeaminase (EC 4.3.1.4), evidence for which has not yet been found in plants. Question marks denote enzymes for which no inferences about compartmentation are yet possible. Abbreviations: THF, tetrahydrofolate; 10-HCO-THF, 10-formyl-THF; 5-HCO-THF, 5-formyl-THF; 5,10=CH-THF, 5,10-methenyl-THF; 5,10-CH_2-THF, 5,10-methylene-THF; 5-CH_3-THF, 5-methyl-THF; DHF, dihydrofolate; Hcy, homocysteine; GSH, glutathione; HM-GSH, *S*-hydroxymethylglutathione; FGAR, formylglycinamide ribonucleotide; FAICAR, formamidoimidazolecarboxamide ribonucleotide; SMM, *S*-methylmethionine; X, methyl acceptor; methyl-X, methylated acceptor.

5,10-methylene-THF reductase, which is required to convert 5,10-methylene-THF to 5-methyl-THF, is confined to the cytosol.

- The cytosol and mitochondria contain cobalamin-independent methionine synthase and can therefore form methionine from homocysteine and 5-methyl-THF. This suggests that mitochondria must import 5-methyl-THF and homocysteine, which is synthesized *de novo* only in plastids[7] and is produced from *S*-adenosylhomocysteine (AdoHcy) only in the cytosol.
- Although plastids carry out all steps in methionine synthesis up to homocysteine, and have a pool of 5-methyl-THF (in its monoglutamyl form, presumably imported)[7], they do not contain cobalamin-independent methionine synthase. Plastids might therefore import methionine from the cytosol. Alternatively, plastids could have a methionine synthase of the cobalamin-dependent-type found in bacteria and animals[7]. This possibility is attractive in light of evidence for the monoglutamyl form of 5-methyl-THF in plastids because the cobalamin-dependent enzyme can use this substrate. However, there are no published plant DNA sequences that encode this enzyme, which is highly conserved between bacteria and animals.
- Neither chloroplasts nor mitochondria have AdoMet synthetase, implying that they import AdoMet from the cytosol. A related deduction, albeit based only on sequence data (the absence of transit peptides), is that these organelles also lack

Table 1. Biochemical and DNA sequence evidence for plant one-carbon metabolism enzymes and their compartmentation

Enzymes[a]	EC numbers	cDNAs cloned[b]	*Arabidopsis* genes[c]	EST search sequences[d]	Refs[e]
(1) 10-Formyl-THF synthetase	6.3.4.3	+	1p	P28723	32,33
(2) 10-Formyl-THF deformylase	3.5.1.10		2x	CAB10517	
(3) 5,10-Methylene-THF dehydrogenase/ 5,10-methenyl-THF cyclohydrolase	1.5.1.5/ 3.5.4.9	+	3p	CAB56756	32–34
(4) Serine hydroxymethyltransferase	2.1.2.1	+	5p	P34899	33,35,36
(5) 5-Formyl-THF cycloligase	6.3.3.2			AW065622	27
(6) Glycine decarboxylase complex					
P-protein	1.4.4.2	+	2p	P26969	37
H-protein		+	2p	P16048–	37
T-protein	2.1.2.10	+	1p	P49364	37
L-protein	1.8.1.4	+	2p	A42494	37
(7) Formaldehyde dehydrogenase (GSH)	1.2.1.1	+	1p	CAA57973	38–41
(8) *S*-Formylglutathione hydrolase	3.1.2.12		1x	AAB84335	41
(9) 5,10-Methylene-THF reductase	1.5.1.20	+	1p	AF181966	13
(10) Methionine synthase (B_{12}-independent)	2.1.1.14	+	2p	CAA58474	42–45
(11) Dihydrofolate reductase/ thymidylate synthase	1.5.1.3/ 2.1.1.45	+	2p	Q05762	34,46
(12) Ketopantanoate hydroxymethyltransferase	2.1.2.11		1x	AAC62893	
(13) *S*-Adenosylmethionine synthetase	2.5.1.6	+	3p	Q96551	42,47
(14) Methionine *S*-methyltransferase	2.1.1.12	+	1p	AAD49574	8,9,48
(15) Homocysteine *S*-methyltransferase	2.1.1.10	+	2p	AF219222	8,[f]
(16) *S*-Adenosylhomocysteine hydrolase	3.3.1.1	+	2p	AAC14714	7
(17) Methionyl-tRNA transformylase	2.1.2.9			AAA58085	49
(18) GAR transformylase	2.1.1.2	+	1p	P52422	50,51
(19) AICAR transformylase/ IMP cyclohydrolase	2.1.2.3/ 3.5.4.10		1x	AAC12842	50
(20) Sarcosine oxidase	1.5.3.1		1x	AAD23888	16
(21) Glyoxylate synthetase	–				10,11
(22) Polypeptide deformylase	3.5.1.31		1x	AAD39667	
(23) Formate dehydrogenase	1.2.1.2	+		Q07511	15–17
(24) Glutamate formiminotransferase	2.1.2.5		1x	AAD20912	
(25) Catalase (peroxidatic activity)	1.11.1.16	+	3p	–	20

[a]Enzyme numbers 1–25 are as in Figure 2. Abbreviations: GSH, glutathione; B_{12}, cobalamin; GAR, glycinamide ribonucleotide; AICAR, aminoimidazolecarboxamide ribonucleotide.
[b]Plus indicates that at least one plant cDNA has been demonstrated by a functional assay to encode the corresponding enzyme activity.
[c]Values are the number of sequenced genes (as of October 1999) in the *Arabidopsis* genome that are highly homologous to *Arabidopsis* or other plant cDNAs (p), or to cDNAs or genes from other organisms (x), which have been demonstrated to encode the corresponding enzyme.
[d]Denotes the sequences used to search dBEST for the EST abundance data of Figure 4. GenBank protein sequences were used in all cases except enzyme 5, for which the open reading frame of an EST was used.
[e]References to biochemical and DNA evidence for enzymes and their compartmentation, and to related reviews.
[f]Bourgis, F. *et al.*, http://www.rycomusa.com/aspp1999/public/cgi-bin/showabs.cgi?0363

AdoHcy hydrolase, and therefore must export AdoHcy to the cytosol for hydrolysis to homocysteine and adenosine. AdoHcy hydrolysis is crucial to the regulation of methylation reactions, because AdoHcy is a potent competitive inhibitor of AdoMet-dependent methyltransferases.

Other deductions from a combination of DNA data and biochemical results are (i) that the two 10-formyl-THF-dependent steps in purine synthesis occur in both mitochondria and plastids, and (ii) that plants have a bifunctional thymidylate synthase–dihydrofolate reductase, not two separate enzymes, and that this enzyme is probably present in both mitochondria and plastids.

Plants have two unique one-carbon reactions and two unusual ones

In terms of plant-specific enzymes, there are only a few major evolutionary novelties in plant C_1 metabolism. The deduced

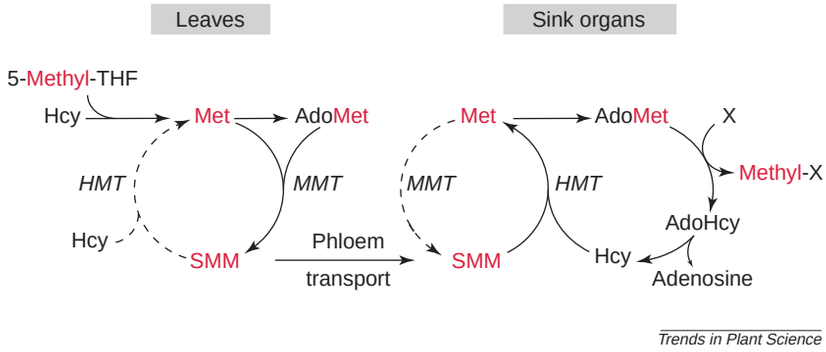


Fig. 3. Long-distance transport of methyl groups via movement of *S*-methylmethionine (SMM) in the phloem. SMM can be formed in leaves by methionine *S*-methyltransferase (MMT), transported in the phloem to seeds or other sink organs, and there reconverted to methionine (Met) by the action of homocysteine *S*-methyltransferase (HMT)[9]. The methionine so formed can give rise to *S*-adenosylmethionine (AdoMet) for use in methylation reactions (X → methyl-X). The hydrolysis of the *S*-adenosylhomocysteine (AdoHcy) produced by these methylation reactions releases homocysteine (Hcy), which can then sustain further conversion of SMM to methionine. Unbroken arrows indicate major fluxes, broken arrows minor ones. The path taken by the methyl groups is indicated in red.

amino acid sequences of most plant C_1 enzymes are strikingly similar to those of other organisms, even though the polypeptides specifying individual activities are sometimes fused together differently. Thus, although other eukaryotes have a trifunctional C_1-THF synthase and plants have a 10-formyl-THF synthetase plus a bifunctional 5,10-methylene-THF dehydrogenase–5,10-methenyl-THF cyclohydrolase[1], the two plant enzymes are clearly homologous to the domains of the trifunctional protein. Likewise, the domains of the bifunctional dihydrofolate reductase–thymidylate synthase are similar to their monofunctional counterparts in other organisms[1]. However, one enzyme is unique to plants, and there is some biochemical evidence for a second unique enzyme. A third enzyme is not known in other eukaryotes; a fourth occurs also in fungi, but its compartmentation is different in plants and it is regulated in an unusual way. These four enzymes are described below.

Methionine S-methyltransferase (MMT)

Methionine *S*-methyltransferase (MMT; Fig. 2, step 14) catalyzes the synthesis of *S*-methylmethionine (SMM), and is unique to plants[8]. SMM can serve as a methyl donor for another methyltransferase, homocysteine *S*-methyltransferase (HMT; Fig. 2, step 15), which converts homocysteine to methionine[8]. (HMT also occurs in other organisms, where it confers the ability to use SMM of plant origin.) MMT and HMT acting in tandem, together with AdoHcy hydrolase, constitute what was thought to be a futile cycle, known as the SMM cycle[8]. However, SMM is now known to be a major amino acid in the phloem, thus the halves of this cycle could be at least partially separated in space and time, with SMM synthesis dominant in leaves and SMM reconversion to methionine dominant in developing seeds or other sinks[9] (Fig. 3). Phloem transport of SMM enables methyl groups to be produced in one place and consumed in another, which emphasizes that some facets of C_1 metabolism must be viewed in a whole-plant as well as in a cellular context.

MMT (Ref. 9) and HMT (F. Bourgis, *et al.*, http://www.rycomusa.com/aspp1999/public/cgi-bin/showabs.cgi?0363) were recently cloned; in both cases extensive use was made of ESTs in the cloning process, and the recombinant enzymes were expressed in *E. coli* for characterization. Interestingly, MMT ESTs are far less abundant than those for AdoMet synthetase and AdoHcy hydrolase (Fig. 4), implying that the major transmethylation flux from AdoMet involves methyltransferases other than MMT. This has been shown in *Lemna*[8], substantiating the idea that useful inferences about metabolic fluxes can be drawn from EST abundance data[5].

Because MMT is an evolutionary innovation, it is noteworthy that it exists as a homotetramer (uncommon among methyltransferases) of an exceptionally large polypeptide with a modular structure[9]. The N-terminal domain shares homology with methyltransferases and has motifs associated with AdoMet-binding. The C-terminal region shares homology with aminotransferases that act on amino acids of the aspartate family, but lacks the conserved lysine residue that binds the catalytic pyridoxal 5′-phosphate cofactor in aminotransferases. Therefore it is likely that the evolution of MMT involved a gene fusion event[9]. The functions of the two domains are unknown.

Glyoxylate synthetase

Glyoxylate synthetase (Fig. 2, step 21) has only been reported in potato tuber chloroplasts[10,11] and has not been cloned. It appears to mediate a THF-dependent condensation of two formate molecules to give glyoxylate, a reaction with no precedent in other organisms. By creating a route from C_1 to C_2 compounds, such an enzyme, in conjunction with aminotransferase and serine hydroxymethyltransferase activities, would enable the elaboration of C_3 (and thence larger) structures solely from C_1 units, as occurs in methylotrophs. Therefore, glyoxylate synthetase is potentially important. The direct reduction of CO_2 to formate is also reported to occur in potato tuber chloroplasts[12]; this reaction is known to occur in anaerobic bacteria but has not been reported in eukaryotes.

NADH-dependent, AdoMet-insensitive 5,10-methylene-THF reductase

The 5,10-methylene-THF reductases (MTHFRs; Fig. 2, step 9) from *Arabidopsis* and maize were recently cloned by genomics-based methods, and characterized by expression in yeast. Both enzymes were found to be NADH-dependent and AdoMet-insensitive[13], unlike the mammalian and yeast enzymes, which use NADPH and are allosterically inhibited by AdoMet – the AdoMet-binding site being in a C-terminal, regulatory domain[14]. The pyridine nucleotide specificity is crucial because the NADPH-dependent MTHFR reaction is physiologically irreversible, because of the large free energy change involved in the reduction of 5,10-methylene-THF and the high cytosolic NADPH:NADP ratio[13,14]. An NADPH-dependent reaction thus commits C_1 units to methyl group synthesis, and potentially can deplete the 5,10-methylene-THF pool; feedback inhibition by AdoMet checks such depletion. By contrast, because the cytosolic NADH:NAD ratio in plants is low, the NADH-dependent MTHFR reaction might well be reversible; this would obviate the need for feedback-inhibition by AdoMet. The NADH-dependence and AdoMet-insensitivity of plant MTHFRs have important implications for the control of C_1 fluxes in plants and their engineering. MTHFRs provide cautionary examples of how proteins sharing high homology can have critically different properties that cannot be predicted from their primary sequences.

Mitochondrial formate dehydrogenase

Formate dehydrogenase (FDH; Fig. 2, step 23) occurs in fungi as a cytosolic enzyme, but both biochemical and DNA sequence data show it to be a mitochondrial matrix enzyme in plants[15]. Moreover, FDH is one of the most abundant soluble proteins in mitochondria from non-green tissues (e.g. up to 9% of the total protein content in potato tuber mitochondria[15]) although it is a minor protein in mitochondria from illuminated, unstressed leaves[16]. FDH is remarkable for being strongly induced in leaves by darkness, by C_1-related compounds, by environmental stresses[16], and in roots by hypoxia and by Fe deficiency[17]. These patterns of developmental and environmental regulation strongly suggest that formate is a major metabolite in certain tissues and conditions.

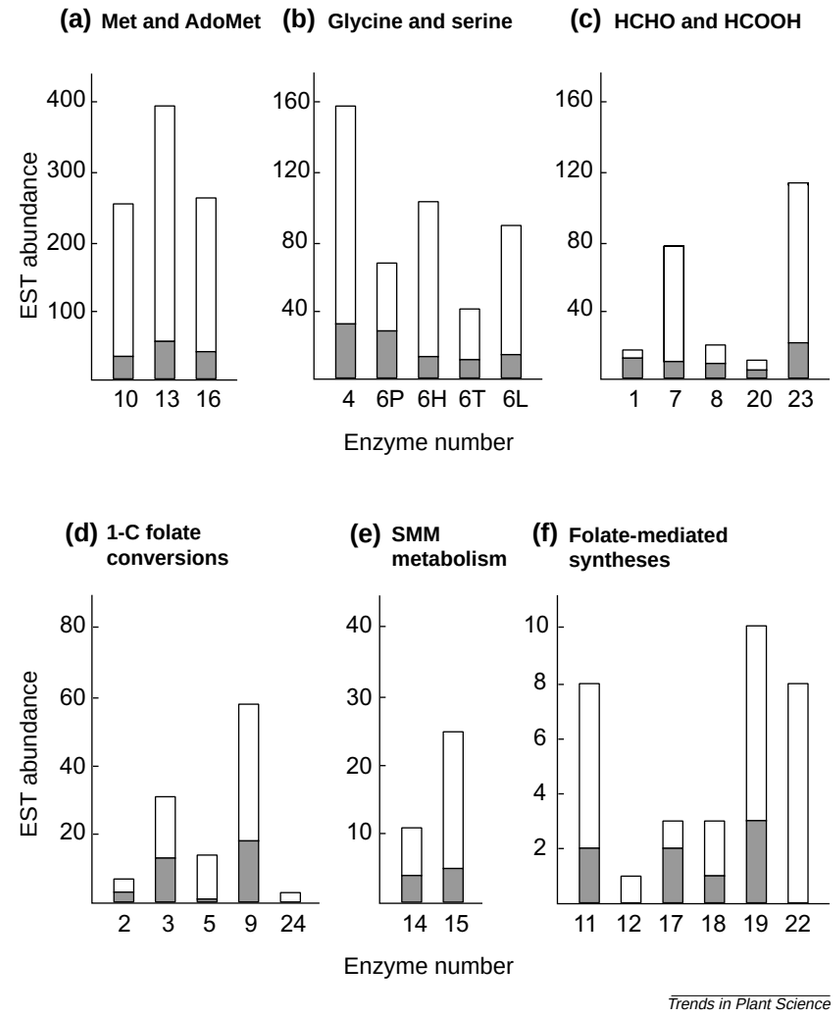


Fig. 4. EST abundance in dBEST for C_1 metabolism enzymes in *Arabidopsis* (gray) and in all other higher plants (white) (as of October 1999). The enzymes, which are numbered as in Figure 2 and Table 1, are divided into functional groups related to (a) methionine and *S*-adenosylmethionine (AdoMet) metabolism, (b) glycine and serine metabolism, (c) formaldehyde and formate metabolism, (d) C_1 substituted folate interconversions, (e) *S*-methylmethionine (SMM) metabolism and (f) folate-mediated syntheses. Note that the EST abundance scales vary by a factor of up to 40 between the six functional groups, but that the abundances of ESTs within each group are generally fairly similar. Note also that the ESTs for methionine and AdoMet metabolism are by far the most abundant, making up 51% of the total. Enzyme 7 (glutathione-dependent formaldehyde dehydrogenase, EC 1.2.1.1) shares ≈50% amino acid homology with alcohol dehydrogenase (EC 1.1.1.1). ESTs were therefore taken to represent EC 1.2.1.1 only if they showed greater similarity to this enzyme (CAA59773) than to an EC 1.1.1.1 sequence (P06525).

Genomic evidence points to unsuspected one-carbon reactions

There is evidence that formate can be formed in the leaves of illuminated C_3 plants as a result of a chemical reaction between glyoxylate and H_2O_2 (Refs 18,19) (perhaps facilitated by the peroxidatic activity of catalase[20]). However, it is not at all clear how formate is produced in the dark, in non-photosynthetic organs, or by C_4 species. Until now, the most plausible route was considered to be from serine or glycine via 5,10-methylene-THF and 5,10-methenyl-THF to 10-formyl-THF, followed by reversal of the 10-formyl-THF synthetase reaction (Fig. 1). Therefore, it is significant that DNA sequence data point to three additional possibilities for the origin of formate that have received little or no attention.

(1) Hydrolysis of 10-formyl-THF: the *Arabidopsis* genome contains two homologs of the *E. coli purU* gene, encoding 10-formyl-THF deformylase. Both the putative PurU proteins appear to be mitochondrial (Fig. 2, step 2). Cognate ESTs indicate that these genes are expressed in *Arabidopsis* and other plants at a modest level (Fig. 4). The 10-formyl-THF deformylase, which in *E. coli* is activated by methionine and inhibited by glycine[21], releases formate from 10-formyl-THF in an essentially irreversible reaction. Therefore, if 10-formyl-THF deformylase is active in plants, it could drive flux out of C_1-substituted folate pools into formate. It is noteworthy that if the C_1 folates were derived via serine and the 3-phosphoglycerate from glycolysis, a flux from C_1 folates to formate (and ultimately CO_2) could operate as an energy-yielding dissimilatory route, bypassing the Krebs cycle.

(2) Oxidation of formaldehyde as its glutathione adduct. The relatively high abundance of ESTs encoding glutathione-dependent formaldehyde dehydrogenase (FADH, also known as class III alcohol dehydrogenase), coupled with the presence of an *Arabidopsis* gene and cognate ESTs that putatively specify *S*-formylglutathione hydrolase (SFGH, also known as esterase D) (Fig. 2, steps 7 and 8) suggest another possible origin of formate. FADH converts the glutathione adduct of formaldehyde (which forms spontaneously) to *S*-formylglutathione, from which formate is released by SFGH. FADH and SFGH are not highly specific enzymes and so might in principle be playing roles unrelated to each other or to formaldehyde oxidation. However, at least for bacteria, this appears not to be the case because FADH and SFGH are members of a preserved operon in many species. There are various possible sources of formaldehyde; these

include spontaneous dissociation of 5,10-methylene-THF formed from glycine or serine[3], and catalase-mediated oxidation of methanol derived from pectin hydrolysis[20,22].

(3) Oxidation of sarcosine or other *N*-methylamino acids. The *Arabidopsis* genome includes a homolog of bacterial and mammalian monomeric sarcosine oxidases, and there are cognate ESTs from *Arabidopsis* and other plants (Fig. 4). Sarcosine oxidase (Fig. 2, step 20) converts sarcosine to glycine and formaldehyde, which can be oxidized to formate. Caution is necessary with this genomic evidence because (a) mammalian sarcosine oxidases also attack pipecolic acid and proline (without release of formaldehyde), (b) a sarcosine oxidase-like enzyme in *E. coli* acts on *N*-methyltryptophan, and (c) it is not clear if there are major sources of sarcosine or other *N*-methylated amino acids in plants. It is therefore significant that sarcosine is a strong inducer of FDH in potato leaves[16], because this suggests that sarcosine is indeed metabolized to formate.

Towards engineering of one-carbon metabolic fluxes

Apart from the photorespiratory flux through glycine and serine[3], little is known about C_1 fluxes in plants. Therefore a major reason to engineer plant C_1 metabolism is to modify the fluxes through the main pathways (Fig. 2), to understand how they are controlled at the enzyme and gene levels[23]. A key part of such work is the use of isotope tracer techniques to measure fluxes[24], and research in yeast[25] that combined genetic modification with ^{13}C NMR analyses of C_1 fluxes provides an excellent paradigm for plants. Although such studies have yet to be made using engineered plants, the utility of ^{13}C NMR techniques in plant C_1 metabolism has been demonstrated with wild-type *Arabidopsis* plants[26,27] and sycamore (*Acer pseudoplatanus*) cell cultures[28]. This work established, *inter alia*, that:

- The glycine decarboxylase complex and the mitochondrial serine hydroxymethyltransferase are tightly coupled via a pool of 5,10-methylene-THF that does not equilibrate with the overall pool[26,28].
- Serine catabolism is probably limited by the THF supply[26,28].
- Plants convert supplied 5-formyl-THF to other C_1 substituted folates[27].

Engineered modification of C_1 fluxes in plants has begun in the area of AdoMet metabolism. When cosuppression was used to reduce AdoMet formation in tobacco, leaf tissue accumulated high levels of free methionine (400-fold that of the wild type) and a catabolic route producing methanethiol was induced[29]. This shows that methionine itself exerts little control over its own synthesis, and supports an earlier finding that plants have an inducible methionine-γ-lyase that can liberate excess methylthio groups[29]. In another approach to lowering AdoMet levels, the bacteriophage T3 enzyme AdoMet hydrolase was expressed in tomato[30] (AdoMet hydrolase converts AdoMet to homoserine and methylthioadenosine). The transformants produced less ethylene (a metabolite of AdoMet), which suggests that AdoMet pools were depleted. Lastly, when AdoHcy hydrolase activity in tobacco was lowered by antisense RNA expression, the plants were morphologically abnormal and showed hypomethylation of DNA (Ref. 31). The most remarkable aspect of these C_1 engineering experiments is that the engineered plants were viable, implying that plants can somehow maintain essential C_1 fluxes in spite of major perturbations.

Future engineering challenges will be to attack folate-mediated C_1 metabolism, for instance by preventing C_1 units derived from formate and serine entering the C_1 folate pool and by stopping methyl groups from leaving it. In addition, the activity of the SMM cycle could be reduced, and the demand for methyl groups increased or decreased by adding or subtracting secondary pathways. Such engineering research, in conjunction with studies of how C_1 gene expression patterns change in the engineered plants, will provide insight into how C_1 fluxes are controlled in plants. To date, we know in a general way that C_1 metabolism is highly regulated at both metabolic and gene levels, and that its capacity can vary greatly as a function of development and environment. However, little is known about either the mechanisms or the extent of this plasticity.

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