Chapter 2

Upstream Metabolic Segments That Support Lignin Biosynthesis

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The synthesis of lignin in differentiating xylem cells places a quantitatively great demand upon the supply of L-phenylalanine. The mechanisms which dictate L-phenylalanine availability are largely unknown. Current knowledge of the upstream metabolic segments that ultimately form L-phenylalanine precursor molecules is reviewed, beginning with the generation of erythrose-4-phosphate and phosphoenol pyruvate from carbohydrate metabolism. Of particular interest is the nature of quinate as a carbon-reserve molecule which seems to have a special precursor relationship with lignin. Possible biochemical interactions between intracellular compartments and different cell types are considered.

The synthesis of lignin occurs in association with the normal developmental process of xylem differentiation; it can also be induced in other tissues in response to the environmental cues of mechanical wounding or infection with pathogens (*1*). The monolignol biosynthetic pathway is a prominent branch of plant phenylpropanoid metabolism, a biochemical network which utilizes L-phenylalanine as a starting substrate (*2*). Up to 60% of the dry weight of higher plants consists of molecules whose precursors once traversed the shikimate pathway to form phenylalanine (*3*), and up to 36% of the dry weight of wood is lignin (*4*). Since the metabolic flux to lignin in xylem-forming cells is vast, the question arises as to how phenylalanine can become selectively available in the correct subcellular spatial locale of one differentiated cell type in response to the demand for lignin biosynthesis? The availability of phenylalanine has been reported to be limiting for phenylpropanoid metabolism (*5, 6*). Phenylalanine is among the most biochemically expensive of the amino acids synthesized by living cells, and the flux to phenylalanine can be expected to challenge cellular reserves of phosphoenol pyruvate (PEP), erythrose-4-P (E4P) and adenosine triphosphate (ATP).

The metabolic *gestalt* (Figure 1) that generates lignin begins with two segments of carbohydrate metabolism which produce PEP and E4P, the starting precursors of aromatic compound biosynthesis. The reserve carbon that initiates carbohydrate metabolism is present as starch in plastids and sucrose in the cytosol. The potential input shown from the Calvin cycle represents a unique capability of photosynthetic chloroplasts. Phenylalanine is one product of the multi-branched, divergent, pathway

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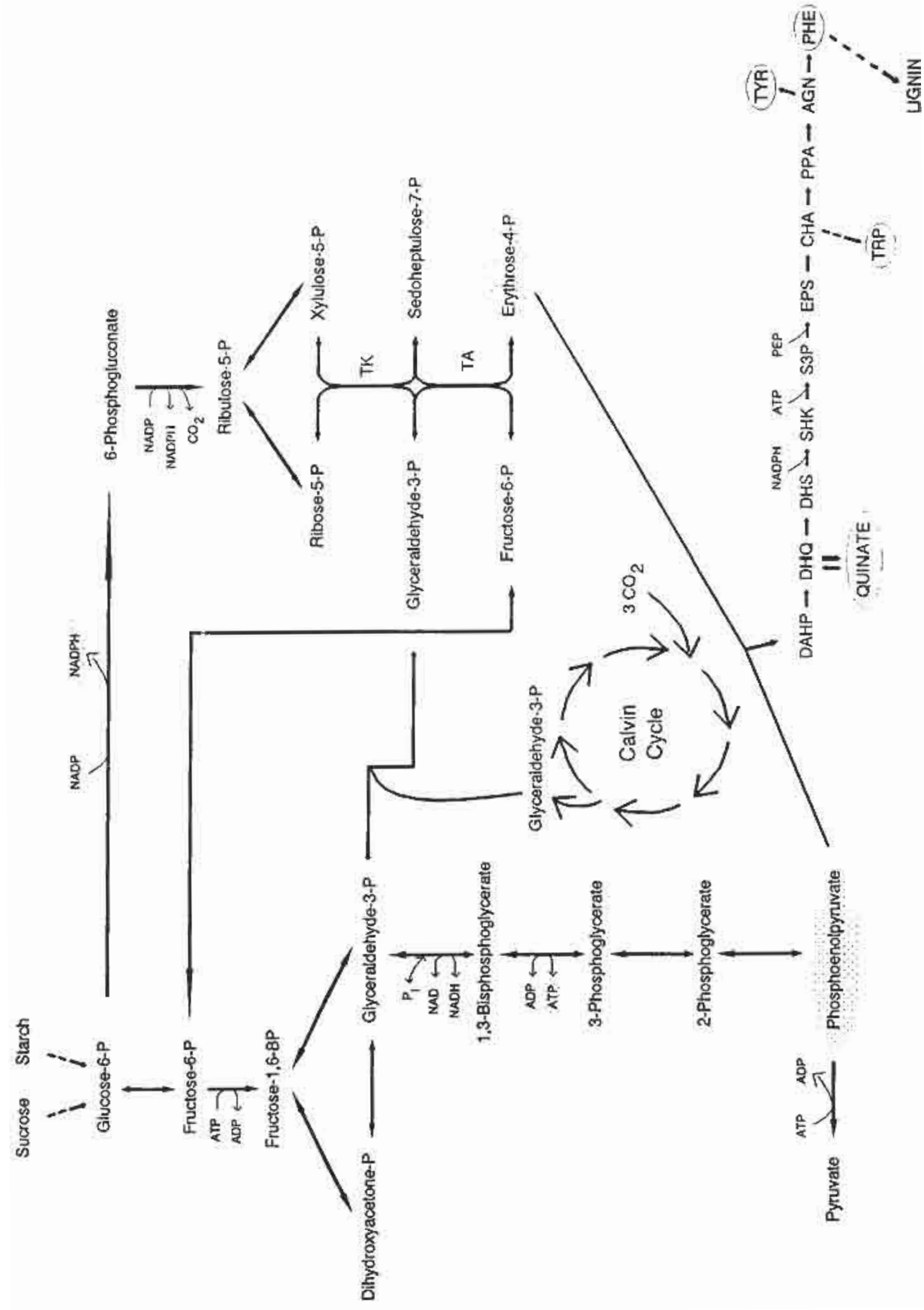
Sucrose
Starch
Glucose-6-P
Fructose-6-P
ATP
ADP
Fructose-1,6-BP
Dihydroxyacetone-P
Glyceraldehyde-3-P
P
NAD
NADH
1,3-Bisphosphoglycerate
ADP
ATP
3-Phosphoglycerate
2-Phosphoglycerate
Phosphoenolpyruvate
ATP
ADP
Pyruvate
NADP
NADPH
6-Phosphogluconate
NADP
NADPH
CO_2
Ribulose-5-P
Ribose-5-P
Xylulose-5-P
TK
Glyceraldehyde-3-P
Sedoheptulose-7-P
TA
Fructose-6-P
Erythrose-4-P
Glyceraldehyde-3-P
Calvin Cycle
3 CO_2
DAHP → DHQ → DHS → SHK → S3P → EPS → CHA → PPA → AGN → PHE
NADPH
ATP
PEP
QUINATE
TRP
TYR
LIGNIN

Figure 1. Relationships of central carbohydrate metabolism to phenylalanine and lignin biosynthesis. Glycolysis, shown at the far left, depicts the pathway present in plastids (see ref. *11* for differences in cytosolic glycolysis whereby various steps can be bypassed under conditions of P_i depletion), while the oxidative pentose phosphate pathway (OPPP) is shown at the upper right. Glyceraldehyde-3-P produced by the Calvin cycle can enter either the glycolytic pathway or the OPPP, as shown. The pathway of aromatic amino acid biosynthesis begins with the condensation of erythrose-4-P and phosphoenolpyruvate to yield DAHP. Compounds maintained within expandable pools are circled (although not circled, considerable pools of SHK may also be maintained). **Abbreviations**: DAHP; 3-deoxy-*D-arabino*-heptulosonate 7-P; DHQ, dehydroquinate; DHS, dehydroshikimate; SHK, shikimate; S3P, shikimate-3-P; EPS, enolpyruvylshikimate-3-P; CHA, chorismate; PPA, prephenate; AGN, L-arogenate; PHE, L-phenylalanine; TRP, L-tryptophan; TYR, L-tyrosine; P_i, inorganic phosphate.

of aromatic compound biosynthesis. The short phenylpropanoid pathway and the monolignol biosynthetic pathway comprise the fourth and fifth segments of the metabolic network. Contemporary reviews typically begin with the last two segments, *e.g.* the excellent review by Dixon and Paiva (*7*). Intensive ongoing research is in the process of elucidating the regulatory mechanisms which partition phenylalanine and its derivatives between lignin and the host of other products formed by competing pathways. Seldom considered has been the question of how phenylalanine availability might be an important variable. In this paper we review aspects of the connecting metabolic networks upstream of the specific pathway to lignin which might dictate phenylalanine availability.

Only a few years ago it was generally thought that the flux of carbon could not be redirected from central metabolism to peripheral metabolic pathways since many complex interactions might be perturbed. Biotechnological approaches were limited to changes that could be made in the specific pathway, *e.g.* mutations to enhance the ability of branchpoint enzymes to outperform competing enzymes, and mutations to abolish regulatory restraints. Although many interventions will undoubtedly severely unbalance metabolism to the detriment of productivity, recent successes in metabolic engineering (*8-10*) have shown that radical changes in the flux into peripheral metabolic pathways can be accomplished by manipulation of central metabolism. The elevated production of aromatic compounds in *Escherichia coli*, over and beyond that achievable by application of conventional approaches, provides one example (*8*). Using a starting background of mutations designed to abolish regulation within the pathway leading to L-tryptophan, additional changes enhancing the flux of E4P and PEP into the pathway were made. Previously unobtained increases in product yield were thus obtained. However, biotechnologists have not been the first to achieve redirection of central metabolism, and we suggest that lignin formation by higher plants may be a premier example.

Segments of Carbohydrate Metabolism

Figure 1 highlights the capacity of the intermeshed oxidative pentose phosphate pathway (OPPP) and the glycolytic pathway to cooperate in the generation of PEP and E4P, respectively. PEP and E4P are the starting precursors needed for phenylalanine (and ultimately lignin) biosynthesis. Glucose-6-P may be mobilized from carbohydrate reserves, namely from sucrose in the cytosol or from starch in plastids. Intact glycolytic pathways exist in both the cytosol and in plastids (reviewed in ref. *11*). These are spatially separate, but cross-compartmental interactions can occur since phosphorylated intermediates such as glucose-1-P, glucose-6-P, dihydroxyacetone-P, 3-phosphoglycerate, and PEP can negotiate the compartmental barrier. The OPPP is present in plastids and is generally considered to be present in the cytosol as well (but see ref. *12*).

Under photosynthetic conditions, glyceraldehyde-3-P (G3P) is potentially available directly from the Calvin cycle for input into the glycolytic and OPPP networks as a precursor for PEP and E4P molecules, as indicated in Figure 1. The ATP and NADPH consumed in the net output of triose-P are produced by the light reactions of photosynthesis.

The sufficient availability of E4P from any cell type in any cell location is a long-standing enigma (*13*). E4P is prone to dimerization because it cannot undergo intramolecular cyclization (*14*). In classical studies of plant cells, levels of E4P were below detection (Bassham, personal communication). D-Fructose-6-P and/or D-sedoheptulose-7-P have been proposed to serve as facile reservoir sources of E4P, thus allowing the rates of synthesis and utilization of E4P to be closely matched (*15*). If Delmer and co-workers are correct in their contention (ref. *16* and unpublished results) that cellulose synthesis requires sucrose as the glucose donor, it would be interesting to know what the impact would be upon E4P production since this would release substantial fructose in cells producing secondary walls.

Availability of E4P and PEP in Chloroplasts

During photosynthesis, G3P output in the chloroplast compartment fuels all subsequent carbon metabolism ($3CO_2 + 9ATP + 6NADPH + 5H_2O \rightarrow G3P + 8P_i + 9ADP + 6NADP^+$). Since G3P utilization by transaldolase (pentose phosphate pathway) and G3P dehydrogenase (glycolysis) would generate E4P and PEP, respectively, abundant resources for direct formation of E4P and PEP in the photosynthesizing plastid would seem to be present. However, uncertainty about the presence of phosphoglycerate mutase and enolase in chloroplasts has raised the question of whether chloroplasts can synthesize PEP (*17*). Furthermore, allocation of G3P to various biosynthetic pathways within the chloroplast is rigorously restrained by complex patterns of regulation for two reasons (*18*). First, five of the six molecules of triose-P formed after three turns of the Calvin cycle must be retained in order to return three 5-carbon molecules to the cycle; over-utilization of triose-P would stop the cycle. Second, export of substantial triose-P from the chloroplast to the cytosol is a mechanism which can guarantee the coupled import of P_i, which in turn is needed to maintain high rates of photosynthesis. Hence, it seems likely that a finely tuned balance is maintained in the photosynthetic chloroplast. Triose-P utilization for low-flux, essential functions in the chloroplast can probably be directly accommodated, but strong selective pressures may exist to shunt excess photosynthate in the form of triose-P to the cytosol to avoid disruption of photosynthesis. Cytosolic triose-P is appropriately mobilized to support metabolism in the various subcellular compartments throughout the plant. This includes products of triose-P metabolism which may subsequently be imported into chloroplasts.

The demand for E4P and PEP as precursors of the fraction of aromatic amino acids used for protein synthesis is quantitatively small, compared to the demand corresponding to the high-flux entry of phenylalanine into the phenylpropanoid channel. Since the aromatic biosynthetic pathway is tightly regulated by a pattern of interacting feedback loops called sequential feedback inhibition (*19*), the amount of E4P and PEP needed for aromatic amino acids that supply chloroplast protein synthesis could be directly derived from G3P photosynthate. The importance of a strictly regulated withdrawal of E4P and PEP has been demonstrated by experimental manipulations where unrestrained entry of E4P and PEP into the aromatic compound pathway of chloroplasts has been shown to disrupt photosynthesis. The normal feedback regulation of 3-deoxy-D-*arabino*-heptulosonate 7-P (DAHP) synthase-Mn (DS-Mn) by L-arogenate was eliminated by use of glyphosate which blocks the accumulation of intracellular L-arogenate (*20*). Geiger and Servaites concluded that photosynthesis was disrupted due to depletion of RuBP for carboxylation (*20*). Interference with P_i import in this case should not be a problem *per se* since the first two reactions of aromatic compound biosynthesis release P_i. However, the exit of shikimate-3-P molecules following glyphosate application may deplete P_i, as well as Calvin cycle carbon in the chloroplast. In addition the generation of P_i in the cytosol might be an important factor in maintaining balanced metabolite exchange between the mitochondrion and the cytosol. The influx of cytosolic P_i (and ADP) into the mitochondrial compartment, coupled with ATP efflux into the cytosol, is essential for continued synthesis of ATP (*21*).

Under any conditions where accumulated chloroplast starch is mobilized as an initial substrate, two molecules of glucose-6-P can enter the OPPP to yield the E4P and NADPH needed for phenylalanine biosynthesis. The fructose-6-P coproduced with E4P can be transformed via glycolysis to yield the two PEP molecules and the one ATP molecule additionally needed as phenylalanine precursors. Operation of this pathway is limited only by the availability of hexose-P and avoids the problem of 5-C depletion since the OPPP can operate freely in a $2C_6 \rightarrow 2CO_2 + C_6 + C_4$ mode, as pointed out by Schnarrenberger *et al.* (*12*).

Availability of E4P and PEP in the Cytosol

Since substantial triose-P is exported to the cytosol in exchange for P_i, this compartment is the logical spatial locale for formation of the substantial amounts of PEP and E4P that are ultimately invested in lignin, as well as in other biochemical spinoffs of phenylpropanoid metabolism. PEP formation would be accommodated by entry of G3P into the cytosolic glycolytic pathway. The presence of a cytosolic OPPP would permit E4P synthesis, and the existence of a complete cytosolic OPPP has been generally accepted for almost 30 years. However, a valid basis for doubts about the presence of all but the initial two dehydrogenases has been raised recently (*12*). This is an important question since the foregoing considerations point to a cytosolic location for the large amounts of E4P needed as precursor for lignin biosynthesis. Perhaps an unknown mechanism exists for E4P synthesis. For example, might E4P be derived via reductive carboxylation of triose-P, as appears to be the case in methanogenic bacteria (*22*)?

Is the Cytosolic Compartment Competent for Phenylalanine Synthesis?

A cytosolic species of DAHP synthase, DS-Co, is ubiquitous in plants, as is CM-2, a cytosolic isoenzyme of chorismate mutase (*23*). In contrast to the tight allosteric regulation of the plastid-localized isoenzymes (DS-Mn and CM-1), DS-Co and CM-2 are completely insensitive to allosteric control. This suggested, as the simplest possibility, the existence of a cytosolic pathway whose output is responsive to substrate availability (*24*). However, the existence of cytosolic enzymes intervening between DS-Co and CM-2 has not been demonstrated. Except for dehydroquinate synthase (step 2), genes encoding all of the common-pathway enzymes that are located in the plastid have been cloned. Use of these genes as hybridization probes has not revealed cytosolic homologs (*25*). However, possible cytosol-localized enzymes might not be homologs of plastid-localized enzymes or might have diverged substantially. Indeed, cDNAs encoding DS-Mn and CM-1 have not been successfully used as probes to obtain the established cytosolic enzymes, DS-Co or CM-2.

As one alternative possibility, we have speculated (*26*) about the existence of a different pathway (phytoshikimate pathway) in the cytosol which does not utilize E4P. This is based upon the ability of DS-Co to use G3P and PEP as co-substrates (*27*). Another possibility raised (*26*) was that DAHP formed by DS-Co in the cytosol might be translocated to the plastid where the plastid enzymes would be used for conversion to chorismate. Transport of chorismate back to the cytosol would effectively provide a mechanism where the main enzymatic machinery of the plastid can be used under conditions where the early-pathway and mid-pathway steps of plastid regulation are bypassed. Flux to phenylalanine would in this way be dictated by initial substrate availability in the cytosol.

Quinate as a Carbon Reserve

Sucrose synthesis is closely coupled with photosynthesis because the accompanying release of P_i in the cytosol is exchanged for plastidic triose-P; a constant supply of P_i is required in the chloroplast for maintenance of photosynthesis (*18*). The utilization of triose-P for starch synthesis in the chloroplast also releases P_i to drive photosynthesis. Quinate synthesis in the cytosol (*vide infra*) can be considered to be entirely comparable to sucrose synthesis in that (**i**) it is a mobile, transportable form of carbon, and (**ii**) its synthesis releases two molecules of P_i (Figure 2). In this context it is of interest that, using both *Brassica* and *Nicotiana* systems, DS-Co levels were induced under conditions of phosphate depletion (*28*). If quinate is indeed the major reserve to be used for those phenylalanine molecules entering the phenylpropanoid pathway, it is an open question whether quinate is transported back to the plastid to be converted to DHQ or SHK (Figure 2) or whether it is metabolized to phenylalanine in the cytosol by unknown steps.

Inducible Bypass Metabolism

The narrative above has indicated that it is highly unlikely that the large amounts of E4P and PEP needed as precursors for phenylalanine molecules destined to enter the phenylpropanoid pathway can be appropriated directly in the chloroplast. It follows that DS-Co in the cytosol must generate the DAHP used in the high-flux flow route. The operation of DS-Co and CM-2 in the cytosol allows various scenarios for compartmental exchange of such molecules as DAHP (or quinate) and chorismate as discussed above.

Modern biochemical advances that allow more meaningful analyses of ever-larger metabolic networks has led to an emerging realization that flux into a given metabolic branch can be modulated significantly by regulation of far-upstream enzymes which deliver substrate. The principle is well illustrated by phenazine pigment production in *Pseudomonas phenazinium* (*29*). These antibiotic agents are formed during the stationary phase of growth from anthranilate, an intermediate of L-tryptophan biosynthesis (Figure 3). During exponential growth, anthranilate is not available to the phenazine pathway because of the finely tuned feedback regulation of the tryptophan pathway. In stationary phase, a second anthranilate synthase (feedback-resistant) is induced along with other enzymes specific to the phenazine pathway. However, this in itself would not provide access to the input of chorismate needed because L-tryptophan and L-tyrosine feedback inhibit the early-pathway formation of DAHP by the two differentially controlled isoenzymes of DAHP synthase present in this group of pseudomonad bacteria. The physiologically-timed expression of a third isoenzyme, insensitive to feedback inhibition, along with the specific phenazine-pathway enzymes establishes unrestrained flux to phenazine that is limited only by substrate availability. Tight feedback control at the branchpoint leading from chorismate to L-tryptophan, L-tyrosine and phenylalanine effectively causes all chorismate formed to be channeled selectively into the phenazine pathway.

Relationship of Quinate and Lignin

The general correlation of quinate levels with lignin content was noted long ago (*30-32*). Progressively greater concentrations of quinate were measured in the comparison of herbaceous angiosperms, woody angiosperms, and gymnosperms. Tracer studies with ^{14}C-quinate demonstrated its active transformation to lignin in the wood of young pine shoots (*33*). These and other tracer studies (*34*) have shown that quinate does not accumulate as a dead-end pool, but rather forms a metabolically active pool with a rapid turnover. Ossipov and Shein showed (*33*) that an inverse relationship exists between quinate accumulation and lignification at the tissue level. Thus, quinate levels in xylem-forming cells of young shoots or in the precambial zone of trunk wood does not exceed 0.5%, which is 10-20 fold lower than in the autotrophic cells of needles. The active utilization of newly formed quinate for lignin biosynthesis accounts for the low quinate levels in xylem-forming cells. In non-lignifying tissues, quinate has alternative fates (*vide infra*). At least in conifer needles, the rate of quinate synthesis exceeds the rate of utilization (*e.g.* for protocatechuate). Quinate accumulates in vacuoles and can be mobilized for active transport throughout the plant. Quinate is extremely water soluble and is transportable in the phloem sap stream (*35*).

Novelty of Shikimate/Quinate Pools in Higher Plants

End-products of biosynthetic pathways such as amino acids, purines, pyrimidines, and vitamins are typically sequestered in metabolic pools of a size appropriate to the range of synthetic demands likely to be encountered. In contrast, intermediary metabolites are not maintained in metabolic pools and are typically present at levels that may be near the limits of detection. Indeed, it has frequently been observed that

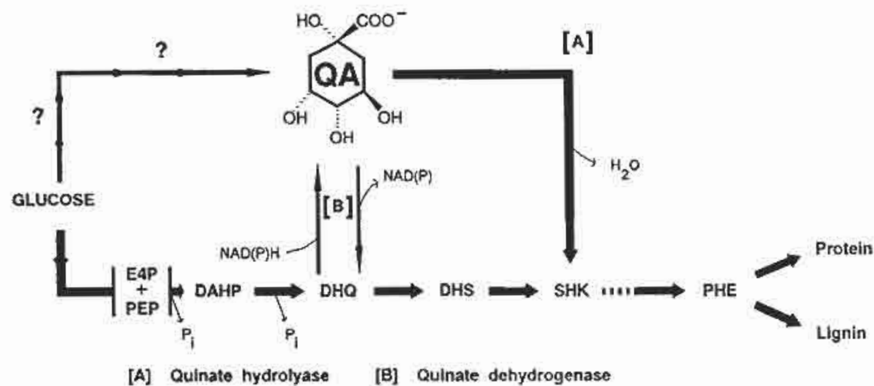

Figure 2. Enzymatic interfacing with quinate (QA). Question marks symbolize the uncertainty of a pathway to QA from glucose which does not involve DHQ. See legend of Figure 1 for abbreviations.

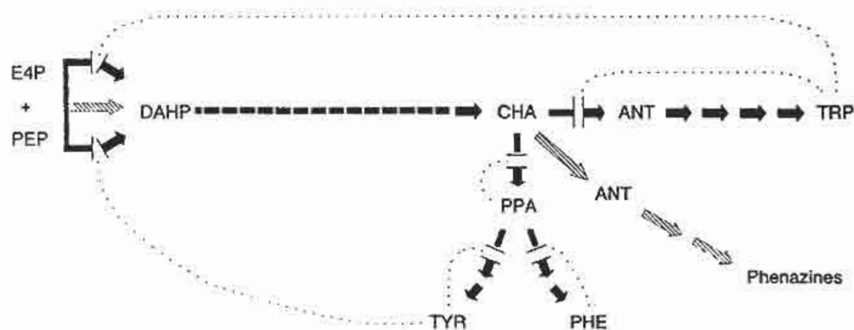

Figure 3. Inducible bypass metabolism in *Pseudomonas phenazinium*. Dotted lines indicate feedback inhibition. Solid arrows represents the enzymatic machinery in operation during primary growth, while shaded arrows identify enzymes selectively induced during stationary-phase metabolism. **Abbreviation:** ANT, anthranilate. See legend of Figure 1 for other abbreviations.

abnormally high levels of intermediates, especially phosphorylated ones, are toxic. Wild-type microorganisms, such as *Escherichia coli* and *Bacillus subtilis*, exemplify this "low-concentration-of-intermediates" rule with respect to shikimate. However, higher plants maintain distinctive pools of shikimate. In addition, the closely related compound, quinate, occupies an even more conspicuous metabolic pool. In angiosperms, shikimate and quinate have been found in most species, with herbaceous plants having low concentrations (*31*) and woody plants having high concentrations (*30*). Gymnosperms possess notably high levels of quinate (*32*), and as much as 10-14% of the dry weight of developing conifer needles is represented by quinate.

Enzymatic Ties to Quinate

Figure 2 is a composite diagram of the reactions which either use quinate as a substrate or form quinate as a product. Quinate dehydrogenase is reversible and potentially can either reduce dehydroquinate to quinate or oxidize quinate to dehydroquinate. Quinate dehydrogenase was one of the first enzymes to be studied in plant cell-suspension cultures (*36, 37*). Quinate hydrolyase, a plastid-localized enzyme, converts quinate to shikimate in corn (*38*) and in pea roots (*39*). In pea roots quinate may supplement aromatic biosynthesis in plastids at the level of shikimate to compensate for the diminution of pathway precursor in the absence of photosynthesis. Quinate hydrolyase has not been observed in conifer needles or xylem-forming cells (*40*). The source of quinate is generally assumed to be dehydroquinate. However, several studies (*41, 42*) have provided suggestive, although not compelling, evidence for the generation of quinate from glucose via a pathway separate from the aromatic biosynthetic pathway. This important possibility has not been evaluated in recent years using the more sophisticated methodology that is now available.

Quinate Metabolism in Gymnosperm Plants

Most of the data about quinate metabolism in coniferous plants comes from the research efforts of Ossipov's group in Russia. *Pinus sylvestris* and *Larix sibirica*, the main organisms studied, have yielded qualitatively similar data. Approaches taken have included rigorous analytical profiles of metabolites in different tissues at various developmental times, tracer studies with radio-labelled precursors, and a limited amount of enzymological characterization. Tracer studies have shown that quinate metabolism is dynamic, with high turnover (*43*). In the autotrophic cells of young needles as much as 10-14% of the total dry weight accumulates as quinate. An active pool of quinate is converted to hydroxybenzoic acids, mainly protocatechuate but some gallic acid as well. A significant portion of protocatechuate is catabolized to TCA cycle intermediates. Most quinate of autotrophic needle cells is maintained in a large vacuolar pool. This pool is a source of quinate for transport to other tissues, notably xylem-forming cells. In xylem-forming cells where lignin synthesis is maximal, quinate content has never been observed to exceed 0.5% of the dry weight (*33*). This correlates with a ten-fold greater specific activity of quinate dehydrogenase than in needles. Accordingly, the lower quinate content has been attributed to its accelerated metabolism by quinate dehydrogenase in order to elevate the precursor pool for lignin.

Quinate dehydrogenase has been purified about 170-fold from both the needles and developing xylem cells of *Larix sibirica* (*44*). Both enzymes were specific for $NADP^+$/NADPH as co-substrate. However, numerous differences in the two enzymes were described. Most strikingly, the enzyme from needles catalyzed an overall conversion of quinate to protocatechuate, and some gallic acid was also formed. Since the overall conversion persisted even after a purification step of affinity chromatography, either a tenacious enzyme complex or a multifunctional protein must exist. Gallic acid might be formed non-enzymatically. In contrast, quinate dehydrogenase from xylem-forming cells did not produce protocatechuate or gallic

acid from quinate. Quinate dehydrogenase from needles, but not from xylem-forming cells, was activated several fold by PEP. The needle enzyme was also uniquely stimulated by Mg^{++}. The enzyme from xylem-forming cells exhibited substantially greater affinities for both quinate and $NADP^{+}$.

Role of Quinate in Bypass Metabolism?

A rough picture is emerging that is consistent with current background information about how quinate metabolism is connected with central carbon metabolism, hydroxybenzoic acid synthesis and lignin synthesis in needles and xylem-forming cells of conifer trees. In needles the high metabolic output sustained by photosynthesis generates enormous quantities of quinate, presumably from dehydroquinate formed in the cytosol. Alternative fates of quinate in the cytosol include conversion to gallic acid (a defense molecule), conversion to protocatechuate and hence to TCA cycle intermediates (perhaps as a central source of carbon during dark metabolism), and deposition within the vacuolar space. Vacuolar quinate is in a state of dynamic turnover and is a transient reservoir for reallocation to the cytosol and for transport to other tissues (notably xylem-forming cells).

Figure 4 illustrates a hypothetical scheme depicting how quinate may participate in a bypass mechanism to provide phenylalanine for lignin biosynthesis. In this scenario lignin output is directly dependent upon both quinate input and the bypassing in the plastid compartment of aromatic-pathway regulation at both the early-pathway and mid-pathway levels. In the plastids of differentiating xylem cells the feedback inhibition of DS-Mn by L-arogenate (*45*) limits DAHP formation in proportion to the demand for aromatic amino acids as substrates for protein synthesis. The metabolic capacity for production of E4P and PEP in non-photosynthetic plastids is probably quite limited. In plastids from pea roots the output of phenylalanine was enhanced by provision of intermediates such as dehydroquinate or shikimate (*46*). Quinate transported from leaf or needle tissue is imported into xylem-cell plastids where quinate dehydrogenase catalyzes the production of dehydroquinate, thus intercepting the common aromatic pathway. Excess chorismate is formed as a result of (**i**) bypassing the initial pathway step which is subject to tight allosteric control, and (**ii**) the tight allosteric control of the main enzymes which utilize chorismate (chorismate mutase-1 and anthranilate synthase). Excess chorismate thus trapped at the branchpoint in the plastid is shunted to the cytosol where an allosterically-insensitive species of chorismate mutase (CM-2) has been demonstrated in many plants (*3, 47*). Two subsequent steps (not yet proven to exist in the cytosol) generate phenylalanine as substrate for ultimate conversion to lignin molecules (or other phenylpropanoid derivatives). An increasing body of information points to the existence of channeling mechanisms that depend upon protein-protein interactions within each of the five metabolic segments considered here (reviewed in ref. *48*). Perhaps dissociable and appropriately regulated complexes exist at the interface of the terminal steps of phenylalanine biosynthesis and initial steps of phenylalanine utilization.

Conclusions

Lignin biosynthesis in differentiating xylem cells is a high-flux process which requires high phenylalanine input. This, in turn, demands a substantial commitment of E4P and PEP. It is unlikely that large quantities of E4P and PEP can be withdrawn from the chloroplast without disrupting photosynthesis. Therefore, the large amount of DAHP fated for conversion to the phenylalanine utilized by the phenylpropanoid pathway must be formed in the cytosol. Quinate is a carbon-reserve molecule that seems to have a special precursor relationship with lignin. We propose that quinate is formed in the cytosol of autotrophic cells as a source of reserve carbon, much of which is destined for lignin biosynthesis. Mobilization of quinate involves transport in the phloem to differentiating xylem cells where it is imported into plastids and converted to dehydroquinate. Entry of dehydroquinate into the shikimate pathway

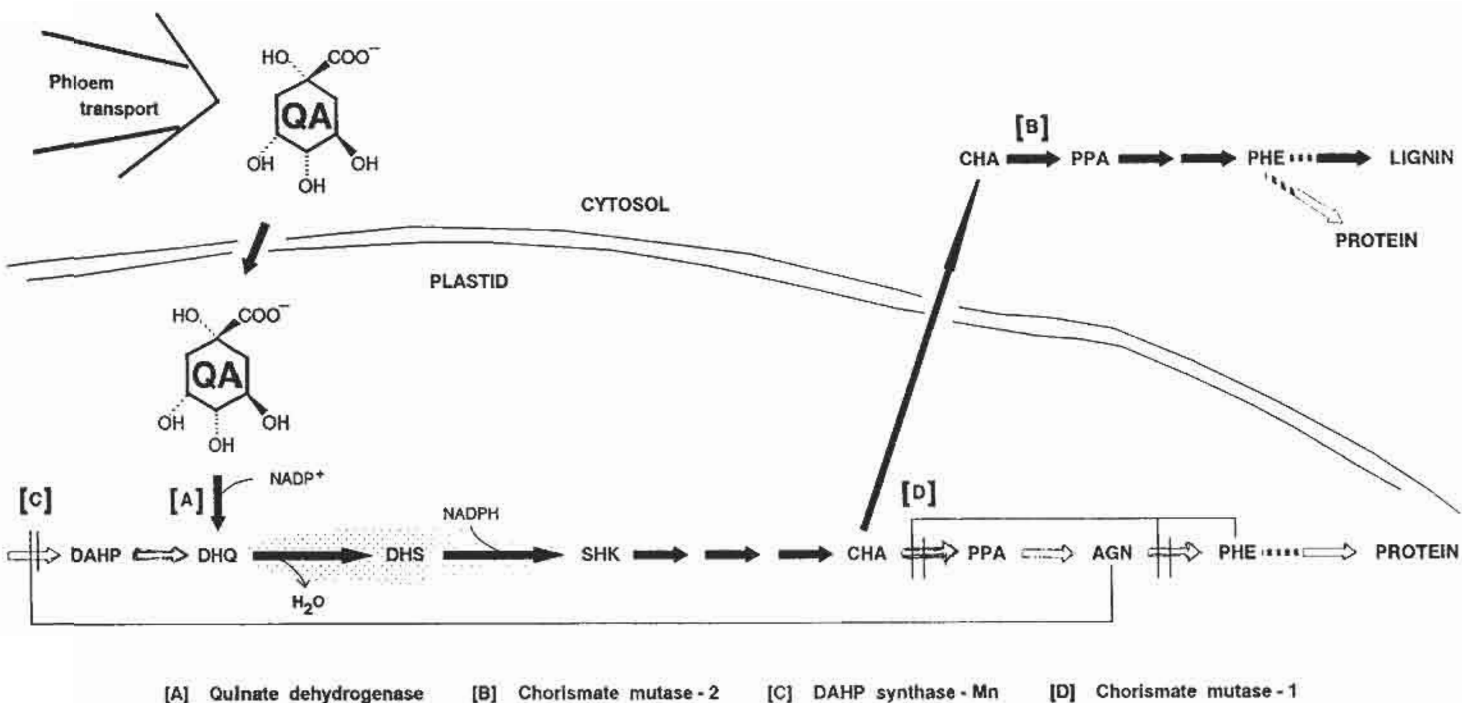

Figure 4. Possible mechanism for utilizing imported quinate (QA) to bypass the regulatory restraints present in the plastids of differentiating xylem cells. Solid arrows show the flow route from QA to lignin superimposed on the regulated steps of phenylalanine biosynthesis (open arrows). The two reactions marked with stippling are domains (dehydroquinase and shikimate dehydrogenase) common to a bifunctional protein.

bypasses early-pathway regulation and forms chorismate in proportion to quinate input. Due to mid-pathway regulation in the plastid, chorismate is exported to the cytosol where reactions are completed to form phenylalanine and then lignin.

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