Annu. Rev. Plant Physiol. Plant Mol. Biol. 1992. 43:241–267

SPATIAL ORGANIZATION OF ENZYMES IN PLANT METABOLIC PATHWAYS

Geza Hrazdina

Institute of Food Science, Cornell University, Geneva, New York 14456

Roy A. Jensen

Department of Microbiology and Cell Science, University of Florida, Gainesville, Florida 32611

KEY WORDS: enzyme organization, plant metabolism, enzyme complex, subcellular localization

CONTENTS

INTRODUCTION

It has been both convenient and productive to treat cells as if they were simply a bag full of enzymes where reactions take place by chance encounter of substrate molecules with enzymes. However, improved methodology is now

0066-4294/92/0601-0241$02.00

generating a basis for suggesting the existence of a strict spatial organization of enzymes in metabolic pathways.

Accumulating evidence indicates the existence of spatially distinct metabolite pools in both prokaryotic and eukaryotic organisms. Even in *Escherichia coli,* where such complexity had not been generally anticipated, tracer experiments showed evidence for metabolic compartmentation of glycolysis during aerobic growth (74). In higher plants, exogenous compounds and endogenous compounds seem to be metabolized in different ways (103). Distinct metabolic pools may be the result of compartmentation of specific pathways in organelles, or of compartmentation accomplished by protein-protein interaction of enzymic components of pathways [i.e. metabolons (102a)] and their association with structural components of cells.

The properties of the individual enzymes in plant cells may be influenced by a multitude of factors. Among these are the interactions between enzymes, with substrates, and with the surrounding solvent. Because of these factors, the concentration of the individual components may affect the activity of the entire cellular environment. Most enzymological studies focusing primarily on the catalytic activity of the individual enzymes are carried out under nonphysiological conditions. Enzymes are usually isolated in an excess of aqueous buffers in which they may be present at nanomolar or subnanomolar concentrations; their activity may be measured over minutes or hours. Under these dilute aqueous conditions functional effects of enzyme-enzyme or enzyme-substrate interactions may not be apparent, except in the case of stable multienzyme complexes or aggregates. In living plant cells proteins and lipids are present at high concentrations, sequestered from the aqueous contents of the vacuole by the tonoplast, and substrates are generally far from saturating. The spatial organization fostered under these conditions promotes enzyme-enzyme and enzyme-substrate interactions believed to be involved in the fine-tuning of metabolic pathways in higher organisms, including plants (91a).

The concept that metabolic pathways consist of sequentially assembled enzymes has only recently been applied to plants. For reviews favoring enzyme complexes see 6b, 59a, 90a, and 104a; for reviews questioning the concept see 6a, 11a, and 121a.

In this selective review, in which no attempt is made to be comprehensive, we emphasize aromatic metabolism and the connecting pathways of carbohydrate metabolism in order to highlight the emerging general evidence for highly ordered spatial organization of plant biochemical pathways.

COMPARTMENTATION OF PATHWAYS IN ORGANELLES

In eukaryotes various structural compartments are closely tied to different aspects of carbohydrate metabolism (103). The enzymes of both glycolysis

and the pentose phosphate pathway are found in both the cytoplasm and plastids; tricarboxylic acid cycle enzymes are located in mitochondria, those of the glyoxylate shunt are in the glyoxysomes; and the enzymes of glycogen metabolism were suggested to be associated with their polymeric substrate, glycogen.

The major biochemical pathway by which both plants and animals utilize carbohydrates is glycolysis. The individual biochemical reactions of glycolysis have been reviewed in detail, their subcellular localization discussed (22).

Role of Isozymes in Metabolic Compartmentation

In plant tissues glycolysis occurs at two different sites: in the plastids and in the cytoplasm (29). Chloroplasts contain the enzymes of the reductive pentose phosphate cycle for the fixation of CO_2. Chloroplasts also metabolize hexoses via the oxidative pentose phosphate pathway, and via glycolysis (29). Isoenzymes catalyzing the same reactions in the glycolytic and oxidative pentose phosphate pathways are also found in the cytoplasm. Cytoplasmic and plastidic isozymes often (but not always) have different physical, catalytic, and regulatory properties. Recent data from *Ricinus communis* show immunological and kinetic differences between cytoplasmic and plastidic isozymes (63a). Their potential for differential function is geared to the specific metabolic demands of the particular tissue. It was reported that cytoplasmic and plastidic pyruvate kinases (PKs) appear to exist as tissue-specific isoforms similarly to that found in animals. Chloroplastic and leucoplastic PKs are immunologically distinct and differ in subunit structure. Cytoplasmic PKs are immunologically similar but exist as either homo- or heterotetramers, depending on the tissue (63b, 91b,c).

Cytoplasmic and chloroplastic isozymes of the first two enzymes of the oxidative pentose phosphate pathway (glucose-6-P dehydrogenase and 6-phosphogluconate dehydrogenase) have been separated and shown to have identical properties, aside from differences in charge (98). The chloroplastic glucose 6-P dehydrogenase is activated by Mg^{2+}-, and this activation was shown to be greater with a bound form of the enzyme than with the soluble form (119). Ribulose bisphosphate at 1 mM concentration completely inhibited the enzyme in the presence of a 2:1 ratio of NADPH:$NADP^+$. Since other intermediates of the pentose phosphate pathway did not inhibit activity, the effect seems to be specific. Illumination of a chloroplast suspension by light resulted in inactivation of the enzyme. This inactivation may be the result of redox control mediated by thioredoxin, because the same effect was caused by dithiothreitol (59). The cytoplasmic isozyme showed similar properties and inactivation, and both isozyme species may be inhibited by a reduction process such as present in the electron transport chain of the thylakoids (3).

While some chloroplastic/cytoplasmic isozyme pairs have similar proper-

ties and differ from each other only in charge, others show large differences. The cytoplasmic and chloroplastic forms of phosphofructokinase have been separated and shown to differ considerably in their kinetic and immunological properties (60, 91b). The cytoplasmic isozyme is activated by high concentrations of P_i; the chloroplastic isozyme is not activated by these conditions. The cytoplasmic isozyme showed also a much higher affinity for its substrate, fructose 6-P, than the chloroplastic species. The plastidic isozyme showed positive cooperativity with fructose 6-P but was inhibited by lower concentrations of phosphoenolpyruvate (PEP) and 2-phosphoglycerate (61). This inhibition was negated by low concentrations of P_i. This positive cooperativity with fructose 6-P and inhibition of the phosphofructokinase when the concentration of PEP and 2-phosphoglycerate increased serves the cells as a convenient regulatory mechanism: Glycolysis is inhibited when the synthesis of starch is activated, and it activates glycolysis again when the synthesis of starch is inhibited (61). Chloroplastic and cytoplasmic isozymes of pyruvate kinase have also been reported in etiolated pea and castor bean plants (54). These isozymes showed different properties, the plastidic form being unstable while the cytoplasmic form remained stable in the absence of reducing agents. cDNAs encoding both cytoplasmic and plastidic PKs have been cloned and sequenced. Deduced primary structures for the respective proteins were shown to be dissimilar (9a). It has been shown (35, 55) that optimal conditions for plastidic isozymes are not optimal for the assay and extraction of the cytoplasmic species. Accurate results about the distribution of both plastidic and cytoplasmic isozymes of glycolysis and the pentose phosphate pathway within the cells requires a thorough appreciation of these differential properties (29). The presence of plastidic and cytoplasmic isozymes of identical enzymic reactions is a clear indication that the glycolytic and pentose phosphate pathways are present in plastids and in the cytoplasm, and that their presence in these two compartments serves different metabolic processes.

Triose-phosphate and glycerate-phosphate are most likely the main products of glycolysis in plastids, and these may be translocated to the cytosol with the aid of the phosphate translocator (34). In the cytoplasm the products of glycolysis are most likely pyruvate and malate (22). Pyruvate is either phosphorylated by pyruvate kinase to PEP or is further transformed in the presence of PEP-carboxylase into oxalacetate. Malate dehydrogenase may then reduce oxalacetate to malate. Malate is either taken up by mitochondria (120) or segregated in the vacuole (37) in free or bound forms.

The pentose phosphate pathway of carbohydrate metabolism seems to have the main functions of producing NADPH for the biosynthetic reactions, providing ribose 5-P for the synthesis of nucleotides, and producing E-4-P for the synthesis of the three aromatic amino acids (22).

The first committed enzyme of the pentose phosphate pathway is glucose 6-P dehydrogenase, which is also present as plastidic and cytoplasmic isozymes (29). The activities of this enzyme are strictly regulated in plants; they are strongly affected by NADPH. The chloroplastic isozyme is sensitive to the ratio of $NADPH/NADP^+$, pH, Mg^{2+}, and substrate concentration (71). The variation in the $NADPH/NADP^+$ concentration ratios may be the controlling factor under in vivo conditions to determine the amount of carbon flow into the pentose phosphate pathway, and hence the production of E-4-P. The availability of E-4-P is a crucial determinant of the extent of carbon flow into the shikimate section of plant aromatic metabolism that produces the aromatic amino acids. The aromatic amino acids may in turn be used by the plant for the synthesis of proteins, or may enter into the multitude of transformations of the specialized branches.

METABOLIC ORGANIZATION IN THE DIVERSE PATHWAYS

Role of Isozymes in Cellular Metabolism

The differential regulatory properties of isozymes can lead to an aesthetically acceptable view of cell metabolism in which isozymes reside at key steps of a metabolic pathway, controlling the flux of intermediate metabolites into the various pathway segments. However, in the case of pathways that are present in multiple compartments, isozyme representation of the entire pathway may occur. In some cases no differences can be found between the components of isozymic systems. Kinetic differences between isozymes alone should not be considered as sufficient support for speculations about their possible regulatory role. All such speculations require that the products of such isozymic reactions be anatomically or otherwise compartmentalized (110).

However, there is ample proof that a number of pathways or parts thereof exist in cells as multifunctional proteins or multienzyme complexes, which permit efficient conversion of substrates by reducing the transit time in the overall reaction sequence. In such an organized system the enzymes are most probably bound together in a manner that permits the channeling of products/substrates from one reaction center to another. The direct transfer of metabolites between the active site of enzymes without their release into the surrounding environment is generally referred to as channeling, and is one of the most prominent features of enzyme complexes (105). Such multienzyme complexes would also exhibit regulatory properties (95, 121).

Carbohydrate Metabolism

The glycolytic pathway is reputed to be the most ancient pathway in cells and also the most centrally located (70). The pathway has been described in most

textbooks as being composed of soluble enzymes. This description arose because most methods used in subcellular fractionation involve homogenization of the tissues and cells under conditions that result in fractional or total solubilization of enzymes. However, investigations in microbial and animal systems indicated that the glycolytic sequence functions in an organized manner under in vivo conditions. The in vivo organization of the glycolytic pathway has been reviewed (78, 89), and only the most salient points are mentioned here.

While the individual enzymes of the glycolytic pathway have been shown to be constituents of multienzyme complexes in both microorganisms and animals, reports of such organization in plants have lagged behind (83a,92). One experimental complication may be that the integrity of enzyme complexes may not survive the harsh conditions of homogenization of plant material, especially the contact with the strongly acidic content of vacuoles.

Less effort has been spent investigating the fine-tuning of glycolytic enzymes in plants than in microorganisms and animals, and this question has not yet been addressed adequately. The description of relatively simple approaches to detect active site–directed enzyme-enzyme interactions (112) may lead to reports on such systems in plants also.

As discussed earlier, PEP is the key intermediate of plant glycolysis that plays a central role in plant metabolism (25). The presence of a plant phosphatase specific for PEP has long been suspected because of the substantial amounts of "PEP phosphatase" found in plants, which interfered with the determination of pyruvate kinase activity. It was proposed recently that PEP phosphatase provides plants with an alternate metabolic route for the conversion of PEP to pyruvate (108). PEP phosphatase has recently been purified from black mustard (*Brassica nigra*) cell-suspension cultures (30). The kinetic properties of this enzyme led to the suggestion that its function in plants is to bypass the ADP-dependent pyruvate kinase reaction during extended periods of phosphate starvation (31).

PEP phosphatase is apparently not the only enzyme in carbohydrate metabolism that responds to low P_i levels in plants. Recent experiments have shown that the extractable activities of pyrophosphate (fructose 6-P-1-phosphotransferase, nonphosphorylating NADP-glyceraldehyde-3-P dehydrogenase, phosphoenolpyruvate phosphatase, and phosphoenolpyruvate carboxylase) increased at least five-fold when *Brassica nigra* leaf petiole suspension cells were subjected to P_i starvation (31). During the same time the activity of the phosphorylating NAD:glyceraldehyde 3-P dehydrogenase decreased about six-fold; the activities of ATP:fructose 6-P-1-phosphotransferase, 3-phosphoglycerate kinase, pyruvate kinase, and NAD malic enzyme were not altered. The activity changes of these glycolytic enzymes (or lack of change) led to the suggestion that an alternate pathway may function in plant carbohydrate metabolism during sustained periods of P_i depletion (31).

Pyruvate and Citric Acid Cycle

Pyruvate is the product of glycolysis. Most pyruvate produced by plant tissues is used as substrate by the tricarboxylic acid cycle compartmentalized in mitochondria (121). Pyruvate dehydrogenase is the first enzyme that interfaces the glycolytic pathway with the tricarboxylic acid cycle. It is a multienzyme complex that consists of pyruvate decarboxylase (thiamine phosphate dependent), dihydrolipoamide acetyltransferase (lipoic acid dependent), and dihydrolipoamide dehydrogenase (FAD-dependent). The three catalytic steps are coupled, channeling the intermediates in an overall reaction yielding acetyl-CoA, CO_2, NADH, and H^+ from pyruvate, NAD^+, and CoA (121).

A second pyruvate dehydrogenase complex is sequestered in plastid compartments. Although much is yet unknown about the detailed structure and composition of these differently compartmented pyruvate dehydrogenase complexes, distinct differences in regulatory mechanisms are apparent (82). Acetyl-CoA is required in the cytoplasm for isopentenyl pyrophosphate (IPP) biosynthesis, separate from the IPP pathway operating in plastids (72). Since the cytoplasmic source of acetyl-CoA is unknown, a third pyruvate dehydrogenase complex located in the cytoplasmic compartment is a possibility.

Another enzyme complex in the tricarboxylic acid cycle is the α-oxoglutarate dehydrogenase (121). The enzyme complex has been isolated from cauliflower buds and was characterized. Its mechanism is similar to that of the pyruvate dehydrogenase complex—differing in product, however, which is succinyl-CoA. α-Oxoglutarate is essential in amino acid metabolism. Plant mitochondria are able to convert it to glutamate by two different mechanisms, either by glutamate dehydrogenase or by a glutamate-oxalacetate transaminase (121).

Seven of the eight TCA cycle enzymes are organized within the mitochondrial matrix where the concentration of proteins is 10 mM or greater. Since diffusion of substrates would be highly restricted under such conditions, the channeling of substrates by sequentially acting enzymes is an attractive scenario. The latter is accommodated in the proposal of Srere et al (104, 104a), which suggests supramolecular organization of the eight enzymes.

Biochemical Interface Between Carbohydrate Metabolism and Aromatic Amino Acid Biosynthesis

PEP and E-4-P originate from glycolysis and pentose-phosphate metabolism, respectively. Differential biochemical aspects of this relationship of carbohydrate metabolism with aromatic amino acid biosynthesis are illustrated in Figure 1.

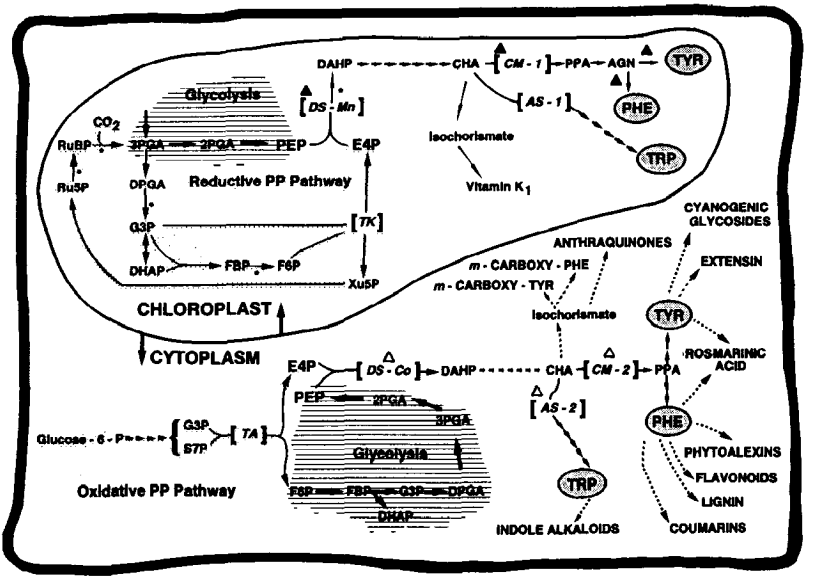

Figure 1 Biochemical juxtaposition of carbohydrate metabolism, aromatic biosynthesis, and secondary metabolism in the chloroplast and cytosol microenvironments of higher-plant cells. Light-activated enzymes are denoted with an asterisk. The three pairs of isozymes participating in aromatic biosynthesis and known to be compartmented as diagrammed are shown within heavy brackets. Solid and open triangles denote enzymes sensitive and resistant to feedback inhibition, respectively. **Metabolite abbreviations**: PP: pentose phosphate; RuBP: ribulose 1,5-bisphosphate; Ru5P: ribulose 5-phosphate; Xu5P: xylulose 5-phosphate; 3PGA: 3-phosphoglycerate; 2PGA: 2-phosphoglycerate; DPGA: 1,3-diphosphoglycerate; G3P: glyceraldehyde 3-phosphate; DHAP: dihydroxyacetone phosphate; FBP: fructose 1,6-bisphosphate; F6P: fructose 6-phosphate; E4P: erythrose 4-phosphate; S7P: sedoheptulose 7-phosphate; DAHP: 3-deoxy-D-arabino-heptulosonate 7-phosphate; CHA: chorismate; PPA: prephenate; AGN: L-arogenate. **Enzyme acronyms**: [TK]: transketolase; [TA]: transaldolase; [DS-Mn] and [DS-Co]: isozymes of DAHP synthase; [CM-1] and [CM-2]: isozymes of chorismate mutase; [AS-1] and [AS-2]: isozymes of anthranilate synthase.

PENTOSE PHOSPHATE PATHWAY/ERYTHROSE-4-P The reductive pentose phosphate pathway is located in the chloroplast and functions during photosynthetic carbon reduction. The enzymes phosphoribulose kinase and ribulose bisphosphate carboxylase are unique to this pathway. Key regulatory enzymes are switched on and off by reduction and oxidation of regulatory disulfide bonds by the ferredoxin/thioredoxin system (13). During photosynthesis, a supply of E-4-P in the chloroplast would be generated by the reductive pentose phosphate pathway as one product of transketolase utilizing 3-phosphoglyceraldehyde and fructose-6-P. Under conditions of light activation the reductive pentose phosphate pathway is thus operational, while in the dark the oxidative pentose phosphate pathway becomes functional.

The oxidative pentose phosphate pathway in the cytosol is switched on and off in response to redox control in a fashion opposite to that of the reductive pentose phosphate pathway, thus avoiding futile cycling. It is believed that E-4-P arises in the cytoplasm mainly as a product of transaldolase utilizing 3-phosphoglyceraldehyde and sedoheptulose-7-P (5).

GLYCOLYSIS/PEP The entire pathway of glycolysis is represented by isozyme pairs that are differentially compartmented in the plastid and in the cytoplasm of higher plants (42). PEP can be derived from glycolysis in both the plastid compartment and in the cytoplasm via enolase, which converts 2-phosphoglycerate to PEP. Data have been obtained, however, that were interpreted to mean that insufficient levels of PEP for aromatic amino acid biosynthesis are generated in plastids, and that a large fraction of PEP is translocated from the cytoplasm (6). C_4 and CAM plants are also known to contain pyruvate phosphate dikinase, which can produce PEP from pyruvate, ATP, and inorganic phosphate. This dikinase has also been detected immunologically in chloroplasts from a number of C_3 plants (4). In gluconeogenic tissues, PEP can be formed by PEP carboxykinase in the presence of ATP.

Aromatic Amino Acid Biosynthesis

A comprehensive, updated review of the biochemistry, enzymology, and regulation of aromatic amino acid biosynthesis in both microorganisms and plants has been published by Bentley (8). In higher plants the shikimate pathway section is present in plastids and has been proposed to exist also as a second pathway in the cytoplasm (48, 57, 58). Thus, like the enzymes of glycolysis and the pentose phosphate pathways, the shikimate pathway section may contain a complete set of plastidic and cytoplasmic isozyme pairs. In the shikimate pathway section of aromatic metabolism (Figure 2) E-4-P formed by the pentose phosphate pathway is condensed with one molecule of PEP that is formed in the last stage of glycolysis by the enzyme enolase to form 3-deoxy-D-arabinoheptulosonate 7-phosphate (DAHP). This reaction is carried out by DAHP synthase (DS) (8, 38). In mung beans, tobacco, sorghum, potato, and pea, distinct isozyme pairs of DS were resolved (56, 57) by ion-exchange chromatography. The properties of the two isozymes were distinctly different. The plastid-localized isozyme (DS-Mn) was stimulated by Mn^{2+}- ions, had a pH-optimum at 6.9, was hysteretically activated by dithiothreitol (DTT), and was saturated at 0.6 mM E-4-P concentration. The cytoplasmic isozyme (DS-Co) exhibited an absolute requirement for divalent cation (Mg^{2+}, Co^{2+}, or Mn^{2+}); was inhibited by DTT; had a pH-optimum at 8.8; and was saturated at 6.0 mM E-4-P concentration. DS-Co also exhibited a broad substrate specificity in which other sugars can replace E-4-P (58).

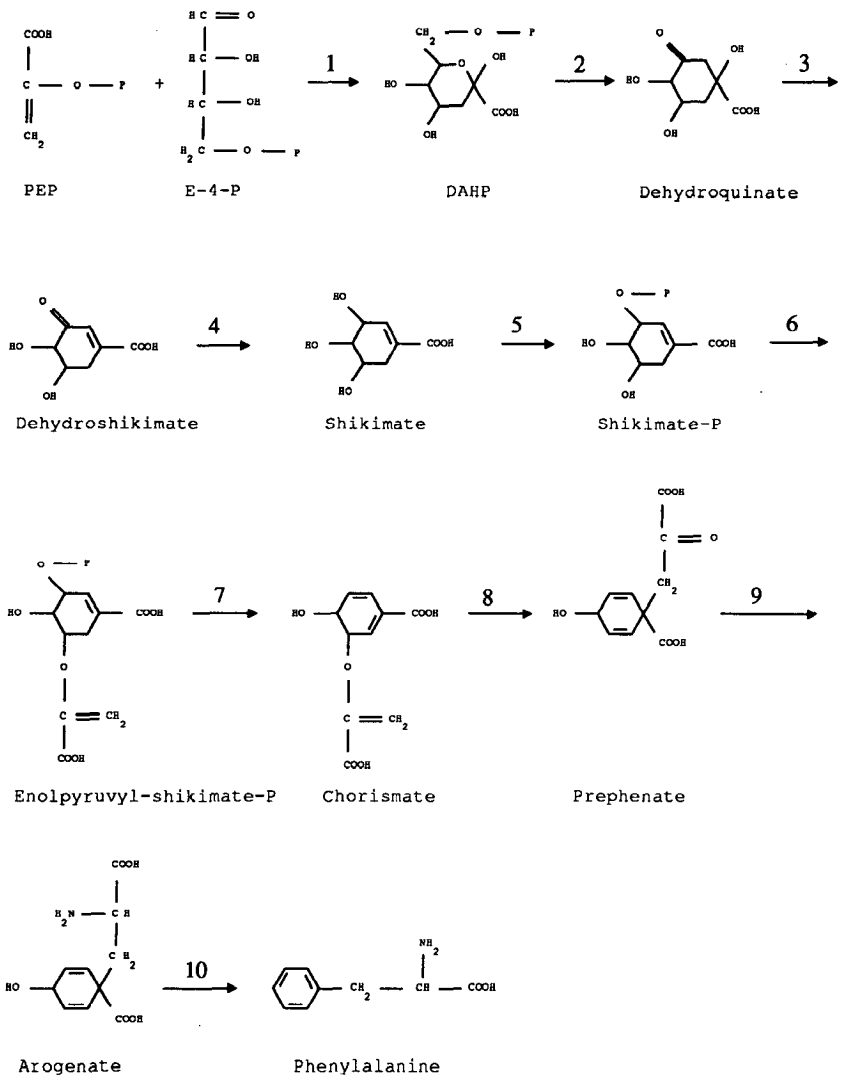

Figure 2 The shikimate section of the plant aromatic pathway. Enzymes of the pathway section are: **1**: deoxy-*arabino*heptonate phosphate synthase; **2**: dehydroquinate synthase; **3**: dehydroquinate dehydratase; **4**: shikimate dehydrogenase; **5**: shikimate kinase; **6**: enolpyruvylshikimate phosphate synthase; **7**: chorismate synthase; **8**: chorismate mutase; **9**: prephenate aminotransferase; **10**: arogenate dehydratase.

Light induction kinetics with *Nicotiana* plants (57) showed that the activity of DS-Mn increased approximately 14-fold during illumination, while that of the Co^{2+}-requiring isozyme remained essentially unchanged. It is striking that the hysteretic redox-activation of DS-Mn resembles that of the key enzymes in the Calvin cycle, which are also redox-activated via the thioredoxin system (13) under conditions of light activation (see Figure 1). DS-Mn showed high sensitivity to allosteric regulation by arogenate whereas the cytosolic DS-Co was insensitive. However, the latter was inhibited by caffeic acid, an intermediate in the phenylpropanoid section of plant aromatic metabolism that leads to the formation of lignin. Mechanical wounding of *Solanum tuberosum* tuber discs, a model system for demonstration of PAL response to environmental signals, was also shown to induce the entire primary pathway of aromatic biosynthesis (84).

The occurrence (40), differential regulation (41), and subcellular location (24) of two distinct forms of chorismate mutase in *Nicotiana silvestris* have been investigated in detail. The one form, designated chorismate mutase 1 (CM-1), was subject to allosteric regulation by the three aromatic amino acids, while the other form, CM-2, showed no allosteric regulatory properties. However, CM-2 was inhibited by caffeic acid, as was DS-Co. The literature on chorismate mutase isozymes in higher plants has been reviewed recently (93).

Isozyme pairs of both DS and CM have now been found in many plant systems (85), suggesting that the plastidic and cytoplasmic organization of shikimate pathway enzymes may be generally similar among higher plants. Tracer studies have indicated that plastid biosynthesis does not account for the total output of plant aromatic amino acids (9). A detailed account of the evidence and rationale supporting the existence of a cytoplasmic pathway of aromatic amino acid biosynthesis has been given recently (58). Although a plastidic and cytoplasmic localization of the shikimate pathway section enzymes seems to be a logical extension from the dual localization of the glycolytic and pentose phosphate pathway enzymes that supply its precursors, clear evidence for most of the cytosolic enzymes remains to be obtained. For example, ultrastructural localization studies of 5-enolpyruvylshikimate-3-P synthase (EPSP synthase) in overproducing tissue cultures of *Corydalis sempervirens* by protein A–gold immunocytochemistry showed that EPSP synthase in the glyphosate-adapted cells was located in the plastids (102). Although extraplastidic EPSP synthase was not detected, success would require cross-reactivity of antibodies. In this connection it is noteworthy that the plastidic and cytosolic isozymes of chorismate mutase present in *Sorghum* were not cross-reactive (101). It is of interest that distinct isozymes of EPSP synthase have been documented (56, 94), but possible differences in sub-

cellular location have not been determined. Two sets of sequences in the *Petunia* genome have been found to hybridize to EPSP synthase cDNA, consistent with the existence of two genes (36). Two genes also seem to exist in tomato (36) and *Arabidopsis* (63).

Two major isozymes of dehydroquinate dehydratase-shikimate: NADP oxidoreductase were reported from pea seedlings; their subcellular localization was identified clearly as plastidic (86). A third, minor isozyme that may be cytoplasmic (87) could not be isolated and localized. The major function of the shikimate section of plant aromatic metabolism in chloroplasts is the production of aromatic amino acids for protein synthesis. Although aromatic amino acids are also needed for protein synthesis in the cytoplasm, they are also used there for the synthesis of such diverse and quantitatively significant natural products as coumarins, lignin, the diverse flavonoid compounds, the isoflavonoid phytoalexins, stilbenes, cyanogenic glycosides, glucosinolates, indole acetic acid, and so on.

Although spatial organization of metabolic networks is undoubtedly universal, it is clear that a diversity of particular arrangements has evolved in nature. In six species of fungi belonging to three classes, steps 2–6 in the shikimate pathway section of aromatic metabolism, which leads to the formation of chorismate, are catalyzed by a pentafunctional protein (1). Although extensive purification of this protein was not carried out, similarities with a purified preparation from *Neurospora crassa* (39), *Saccharomyces cerevisiae* (28), and *Aspergilus nidulans* (16) were noted. This pentafunctional protein was reported to channel intermediary metabolites (26, 117) and to show coordinate activation by the first substrate, DAHP (118). Such enzymic arrangement in cells would have a novel regulatory function, directing the flow of metabolites in response to both substrate availability and end product levels.

Results from chemical modification and limited proteolysis experiments showed that the first two activities of the pentafunctional enzyme were spatially distinct. While fewer experimental data were available for the other three enzyme functions of the *arom* pentafunctional enzyme, they were consistent with the suggestion that the activities occur at separate sites on the polypeptide (18, 19), a conclusion confirmed by recent work (32). Coggins et al (19) have questioned the existence of the "coordinate activation" or "catalytic facilitation" on the basis that the preparations used to demonstrate these phenomena were crude, appeared to have different kinetic parameters from those reported for well-characterized, homogeneous preparations, and were most likely damaged by the presence of proteolytic enzymes (18). However, in this connection it is interesting that Byng et al (14) presented evidence in crude extracts of *Euglena gracilis* that was consistent with coordinate activation of the *arom* protein by DAHP, the initial substrate.

In higher plants dehydroquinase and shikimate dehydrogenase appear to

coexist on a bifunctional protein (11, 64, 86). The remaining three enzymes corresponding to the *arom* pentafunctional protein have been identified as monofunctional enzymes in higher plants (87). These have all been reported to be located in the chloroplast compartment. The monofunctional, plastid-localized EPSP synthase, of course, has been one of the most intensively studied plant enzymes owing to its identification as the herbicidal target of glyphosate (2). Perhaps an *arom* protein exists in the cytoplasm that catalyzes reactions intervening between the cytoplasmic DS-Mn and CM-1? Higher plants may possess an *arom* protein in which absolute channeling occurs and individual catalytic steps cannot be monitored. Such an *arom* protein would not be recognized without special effort. It is suggestive that *Euglena gracilis*, which possesses the higher-plant pattern of tyrosine and phenylalanine biosynthesis (15), is known to possess an *arom* protein (90).

The branch of tryptophan biosynthesis is the segment of the aromatic pathway that has received the least attention. The existing literature has been reviewed by Poulsen & Verpoorte (93). It is noteworthy that *Euglena gracilis*, a photosynthetic eukaryote, possesses either a single protein or a tightly associated complex of catalytic domains corresponding to the last four steps of tryptophan biosynthesis (97). In addition, the nonidentical subunits normally present in anthranilate synthase (amidotransferase) were found to be fused in *Euglena* (45, 46). In higher plants most enzyme studies have focused upon anthranilate synthase (see Figure 1), where a feedback-sensitive isozyme (AS-1) and a feedback-resistant isozyme (AS-2) were localized in the plastids and in the cytoplasm, respectively (12).

Phenylpropanoid and Flavonoid Pathways

The enzymic organization in the phenylpropanoid and flavonoid pathway sections were discussed in reviews covering the literature through 1980 (106, 107). We discuss only selected data that have appeared since the publication of these reviews.

The aromatic amino acids in plants apparently do not enter into general pools. Feeding experiments with labeled phenylalanine in buckwheat seedlings indicated that approximately 20–25% of the label appeared in metabolic end products of the aromatic pathway (75).

The general phenylpropanoid pathway (Figure 3), as well as the diverse flavonoid branches, are localized in the cytoplasm. Although older reports of fractional amounts of phenylalanine ammonia-lyase (PAL) being associated with plastids were carried over into fairly recent books (e.g. 73), PAL and chalcone synthase, the first enzyme of the flavonoid pathway section, are clearly cytoplasmic (50). They also exist in multiple isozymic forms. Two-dimensional gel electrophoresis of in vitro translation products in *Colletotrichum lindemuthianum*–infected bean seedlings showed the presence of

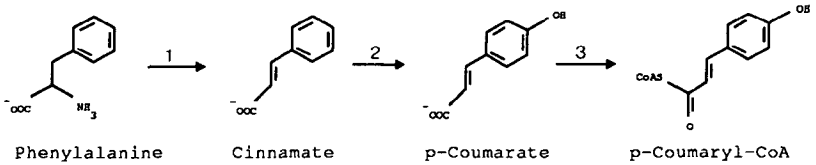

Figure 3 The general phenylpropanoid section of the plant aromatic pathway. Enzymes in the pathway section are **1**: phenylalanine ammonia-lyase, **2**: cinnamate 4-hydroxylase, **3**: p-coumarate:CoA ligase.

characteristic sets of PAL and chalcone synthase isozymes in directly infected tissues that produced specific isoflavonoid phytoalexins (44).

The first enzyme of phenylpropanoid metabolism is PAL, which deaminates phenylalanine to cinnamic acid. PAL is a tetrameric enzyme with a subunit M_r of approximately 83,000 (47), although subunit M_rs of 77,000, 70,000, and 53,000 were also reported from French beans (*Phaseolus vulgaris* L.) (10). PAL is present in plants as multiple isozymes that differ in K_m values and pI of the subunits (10). The expression of the enzyme is under the control of a gene family and is regulated differently in the different plant organs (43) upon stress conditions and illumination. The induction of the diverse PAL isozymes upon different stimuli and in different tissues suggests a strict organization not only of this enzyme in plants but also of the general phenylpropanoid pathway section.

Cytological investigations using sucrose density gradient fractionation of cell contents have indicated the association of key phenylpropanoid and flavonoid pathway section enzymes with the endoplasmic reticulum (ER) membranes in *Hippeastrum* petals (113). A close association of PAL with the second enzyme in the phenylpropanoid pathway section, cinnamate 4-hydroxylase, an ER-embedded enzyme, was also shown in channeling experiments in cucumbers (23) and buckwheat seedlings (49). These data show that at least the phenylpropanoid section of the plant aromatic pathway, or parts thereof, exist as a consecutively assembled, membrane-associated enzyme complex, as suggested in an earlier review (107). In such a complex pathway intermediates are channeled between the individual enzymes and are not released into the cytosolic environment (49). This is consistent with the observation that, unlike the chloroplastic shikimate pathway section that is under strict allosteric control, the cytoplasmic counterpart and the downstream phenylpropanoid and flavonoid sections of aromatic metabolism are not subject to feedback control.

Although feedback control of PAL by cinnamic acid has been suggested (79), the concentrations of cinnamic acid used were not physiological. As discussed above, cinnamic acid is channeled between PAL and cinnamate

4-hydroxylase in the living cells and is not released in the surrounding cytosol. Therefore, the observed inhibition may have been caused by nonspecific effects of *trans*-cinnamic acid on plant cells (116).

Metabolic compartmentation that prevents the diffusion of intermediary metabolites and permits the simultaneous formation of a number of different metabolic end products may be accomplished by enzyme complexes associated with structural elements of the cell (48). It has been shown that chalcone synthase, the key enzyme of the flavonoid pathway section, which was previously thought to be cytosolic, is associated with ER membranes in vivo (51). This evidence came from cell fractionation experiments using buckwheat hypocotyl tissue on sucrose density gradients, where the activity of chalcone synthase, the first enzyme of the flavonoid pathways (Figure 4), coincided with that of the cinnamic acid hydroxylase, both in the presence of $MgCl_2$ and EDTA. These enzymological data were confirmed by immunoblotting the gradients and by electron microscopic immunocytochemical investigations both in situ and with isolated membranes. The data clearly indicate association with cytoplasmic structures. Although an investigation from another laboratory using spinach leaves came to a different conclusion (7), a closer study of the electron micrographs suggests structural association of chalcone synthase in this plant species also.

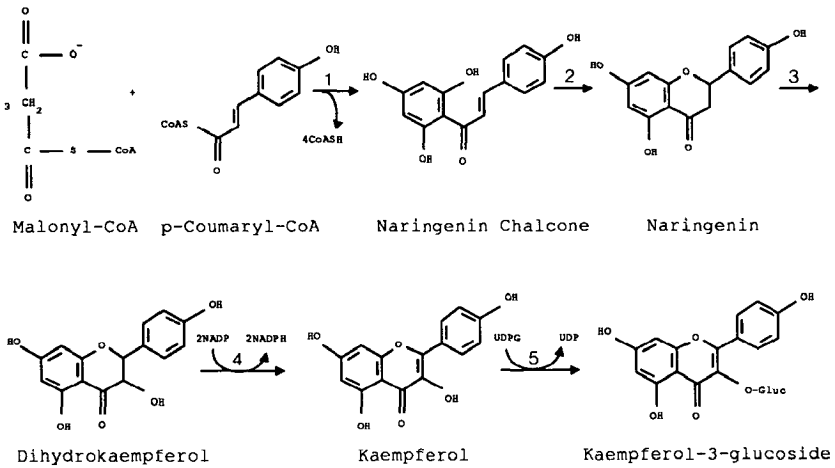

Figure 4 Flavonoid section of plant aromatic metabolism leading to the formation of kaempferol-3-glucoside. Enzymes in the pathway section are **1**: chalcone synthase, **2**: chalcone isomerase; **3**: flavanone 3-hydroxylase; **4**: 3-hydroxyflavanonol oxidase; **5**: flavonol 3-glucosyltransferase.

Cyanogenic Glycosides

Cyanogenesis is the process by which living organisms produce HCN (20). A number of higher plants, including important crop plants such as sorghum, cassava, and white clover, produce and accumulate cyanogenic glycosides, the precursors of HCN. The formation of HCN is a two-step process in plants. The first step is the hydrolysis of the β-glucosidic bond to form the hydroxynitrile and glucose. The hydroxynitrile than undergoes a chemical dissociation to yield HCN and an aldehyde (20).

The biosynthesis of cyanogenic glycosides is by now well understood. An amino acid is hydroxylated to form an N-hydroxylamino acid, which is then converted to an aldoxime, this in turn to a nitrile. The nitrile is hydroxylated to form an α-hydroxynitrile, which is glucosylated to form the corresponding cyanogenic glycoside (80).

Stereochemical studies on the biosynthetic mechanism of cyanogenic glycoside formation indicated the involvement of a monooxygenase in the hydroxylation step. In later studies a cell-free particulate fraction was obtained from dark-grown sorghum seedlings that catalyzed not only the formation of the p-hydroxyphenylacetonitrile but also the multistep conversion of tyrosine to the p-hydroxymandelonitrile, the last step before glucosylation in the biosynthetic pathway (80). When the particulate enzyme system and a sorghum UDP-glucosyltransferase were incubated with tyrosine and NADPH, the cyanogenic glycoside dhurrin was obtained (80). The maximum rate of oxidation of p-hydroxyphenylacetonitrile to p-hydroxyphenylmandelonitrile of the sorghum preparation was less than one third of that observed for p-hydroxyphenylacetaldoxime when it oxidized the same product (99). Trapping experiments have shown that the aldoxime is converted first to p-hydroxyphenylacetonitrile before it undergoes a subsequent hydroxylation. Therefore, the preferential oxidation of the nitrile produced in situ must be the result of cooperation between the two enzymes. N-Hydroxytyrosine and tyrosine were utilized in a similar manner, showing that both compounds are converted to p-hydroxymandelonitrile by the membrane-bound enzyme preparation at rates much greater than that found with p-hydroxyphenylacetonitrile (20). The production of dhurrin from tyrosine by the particulate enzyme system from sorghum is clear evidence for channeling in the cyanogenic glycoside biosynthetic pathway in a similar manner.

Isochorismate Derivatives

Figure 1 illustrates a specialized pathway network that branches off from a mid-pathway intermediate of aromatic amino acid biosynthesis. Chorismate is rearranged in one step to produce isochorismate, a metabolite whose full significance in higher plants is just now becoming known (93). Anthraquinones, which can accumulate to substantial levels in the vacuoles of

plants, have been shown to originate from isochorismate (100). On the other hand, isochorismate is also the precursor of the quantitatively minor vitamin K_1 (phylloquinone), which is located in the chloroplasts. The presence (53, 93) of two chorismate synthase isozymes—one located in the chloroplast and one in the cytoplasm—would provide a mechanism that would accommodate the different quantitative outputs of vitamin K_1 and anthraquinone, as well as the different partitioning of the products in separate compartments.

Some higher plants (e.g. *Iris*) produce *m*-carboxy derivatives of phenylalanine and tyrosine. As illustrated in Figure 1, these originate from isochorismate (66, 67). Enzymes intervening between isochorismate and the *m*carboxy amino acids have not yet been identified. Zamir et al (122) have speculated that isoprephenate, *m*carboxyphenylpyruvate, and *m*carboxy-hydroxyphenylpyruvate are formed by enzymes that catalyze analogous reactions to those of chorismate mutase, prephenate dehydratase, and prephenate dehydrogenase. An alternative possibility is that isoprephenate and isoarogenate are interconverted by transamination and that isoarogenate is then converted to *m*carboxy-phenylalanine and *m*carboxy-tyrosine by enzymes that parallel arogenate dehydratase and arogenate dehydrogenase, respectively. The current difficulty in identifying these enzymes may well reflect some mechanism of spatial organization such as channeling.

Other Specialized Pathways

In contrast to the consistency of fundamental features of pentose phosphate metabolism and aromatic amino acid biosynthesis in higher plants as diagrammed in Figure 1, the qualitative and quantitative diversity of secondary metabolism is truly striking. Thus, the network of biochemical systems that connect with the aromatic amino acid end products in the cytoplasm are highly variable from plant to plant. Most of these specialized pathways appear to be subject to induction or elicitation by environmental, physiological, or developmental cues. In many cases the general outline of a specialized pathway has been deduced from tracer experiments and precursor studies, but full documentation at the enzymological level has been difficult. It is likely that, in parallel with the precedent of cyanogenic glycosides, these difficulties will prove to be related to the existence of channeling mechanisms. In a single plant species numerous specialized pathways may exist in competition for cytoplasmic aromatic amino acids, most notably the phenylpropanoid and flavonoid pathways. In the following narrative, a small sample of specialized pathways is given to illustrate systems where future details of compartmentation or other spatial organization of catalytic domains can be anticipated.

ALKALOIDS Higher plants produce alkaloids from all three aromatic amino acids. Tryptophan decarboxylase, which catalyzes the initial step in indole

alkaloid synthesis in plants such as *Catharanthus roseus*, is a pyridoxoquinoprotein (91). In cases where a high output of indole alkaloids occurs, an understanding of the following aspects is essential: (*a*) the energy-drain impact of hyperutilization of a quantitatively minor amino acid that requires more energy input for biosynthesis than any of the protein amino acids; (*b*) the nature of cross-pathway relationships with respect to the role of tyrosine as a precursor of the pyrroloquinoline quinone cofactor needed for tryptophan decarboxylase; and (*c*) the spatial and metabolic relationship to the pathway of auxin synthesis, which also utilizes tryptophan as the starting precursor. In the latter case the synthesis of indoleacetic acid from L-tryptophan has been concluded to occur in the cytoplasm (97).

Isoquinoline alkaloids are derived from L-tyrosine. The initial catalytic step has been shown to be L-tyrosine decarboxylase, an enzyme that can be induced in the presence of a fungal inhibitor (77). The extreme instability of tryptophan decarboxylase may reflect currently unknown protein-protein interactions involved in pathway organization.

Tropane alkaloids are derived in part from phenylalanine in species of *Datura stramonium* (69). The aromatic ring of tropane alkaloids is derived from β-phenyllactic acid, which is formed from phenylalanine. The remainder of the molecule is derived from ornithine or arginine. Most of work in this system has been done with tracer and precursor feeding experiments, and little information is available at the enzyme level. The nature and mechanism whereby a specialized pathway such as that leading to tropane alkaloids interacts in competition for phenylalanine with the multitude of specialized phenylpropanoid and flavonoid pathways derived from cinnamic acid should be of great interest.

EXTENSIN Among glycoproteins located in primary cell walls of higher plants is extensin, a hydroxyproline-rich glycoprotein. In some dicotyledons extensin can make up as much as 10% of the dry weight (65). L-Tyrosine comprizes about 13% of the amino acid composition of extensin. The details of the assembly of extensin and the mechanism of its final spatial organization in intimate association with the cell surface remain to be determined. A portion of the ultimate elucidation will concern the spatial relationship of the substantial pool of L-tyrosine required to support extensin synthesis.

END PRODUCT CONJUGATES Many secondary metabolites are formed as conjugates of molecules that represent different sections of aromatic biosynthesis. Thus, chlorogenic acid (a well-known component of potato tissue) is a conjugate derived from quinic and caffeic acids. The presence of caffeyltyrosine and caffeyltryptophan has been reported in coffee beans (17). Figure 1 illustrates the case of rosmarinic acid, which is derived from the equimolar

input of phenylalanine and tyrosine. De-Eknamkul & Ellis (27) have suggested that the striking similarity in properties of PAL and tyrosine aminotransferase may reflect their spatial proximity in a microenvironment dedicated to rosmarinic acid biosynthesis. The formation of conjugates requiring a balanced input of metabolites representing a given section of aromatic metabolism may reflect a spatial relationship of aromatic pathway segments.

PHENYLPROPANOID DERIVATIVES In addition to the specialized phenylpropanoid and flavonoid pathways discussed, a wide diversity of other specialized pathways diverge from the main phenylpropanoid pathway—e.g. lignin and coumarins. The differing specialized pathways respond to different environmental cues such as mechanical wounding, UV light, osmotic stress, and fungal elicitors. Since all of these pathways share early phenylpropanoid pathway steps such as that of PAL in addition to the unique pathway steps, a dilemma exists whereby a mechanism must exist for PAL to respond to all inductive signals in contrast to the specificity of elicitation for later enzyme steps. Extensive molecular genetic documentation demonstrates a large gene family for an enzyme like PAL, which plays a crucial role in the multiple divergent pathways. However, surprisingly little information is available at the enzyme level about differential protein-protein interactions and subcellular location in which specific genes are related to specific PAL isozymes. It seems likely that different isozymes (and perhaps particular isoform variants of each) possess specific spatial relationships with enzymes of the different specialized pathways. A mechanism would then exist that would dedicate fractional properties of total PAL catalytic potential to the numerous specialized branches that diverge from the common phenylpropanoid trunk.

Other Amino Acid Pathways

GLYCINE DECARBOXYLASE The glycine decarboxylase multienzyme complex is a key unit of the photorespiratory C-2 cycle of the C_3 plants (88). The complex catalyzes the conversion of glycine in the presence of NAD^+ and tetrahydrofolate into carbon dioxide, ammonia, NADH, and N5 N^{10}–methylenetetrahydrofolate. The complex is located in the mitochondria, where it exists at concentrations of up to 130 mg protein/ml. The glycine decarboxylase complex consists of four different protein components (115). These are the 100-kDa P-, the 13.9-kDa H-, the 45-kDa T-, and the 59-kDa L-proteins. The P-protein binds pyridoxal 5-P, which forms the initial Schiff base with the α-amino group of glycine. The methylamine moiety remaining after the loss of the α-COOH of glycine binds to the H-protein, which shuttles it to the T-protein for transfer to tetrahydrofolate to form methylene tetrahydrofolate. The last reaction step is the oxidation of the dihydrolipoamide

group of the H-protein with sequential reduction of FAD and NAD^+, which is carried out by the L-protein.

The glycine decarboxylase complex constitutes approximately one third of the soluble protein in pea mitochondria. In the mitochondria the complex has 2P-dimer : 27H-monomer : 9T monomer : 1L-dimer composition and is stable (88). Upon dilution it dissociates into its component enzymes, preventing purification by gel filtration or ultracentrifugation. The dissociated form of the complex that occurs at low protein concentrations has different properties from that of the intact complex. In the dissociated form the reaction rate is much lower, being limited by the rate of H-protein diffusion, which contains the lipoamide cofactor and acts to shuttle reaction intermediates among the P-, T-, and L-proteins. At higher protein concentration the complex reassociates rapidly and does not require the H-protein to shuttle reaction intermediates among the protein constituents.

GLUTAMINE SYNTHETASE Glutamine is an important amino acid whose synthesis proceeds in different spatial locations. Glutamine serves as a major nitrogen transport compound in many plants, including the garden pea (*Pisum sativum* L.) (111). Glutamine is synthesized by the enzyme glutamine synthetase, a structurally complex enzyme. Glutamine synthetase activity increases dramatically in the cotyledons, where large amounts of glutamine are synthesized for nitrogen transport to the developing seedling (68). In *Pisum* the glutamine synthetase is present both in the chloroplasts and in the cytoplasm. Chloroplast and cytoplasmic isozymes are encoded by homologous nuclear genes that are differentially expressed in vivo (109). One of the cytoplasmic glutamine synthetase isozymes is accumulated in nitrogen fixing nodules and cotyledons, where large amounts of nitrogen have to be assimilated and transported to the shoots and roots. This isozyme serves to synthesize glutamine for intercellular transport. The other cytoplasmic isozyme has been detected in nitrogen fixing nodules only, where it may function in glutamine synthesis for intracellular use, assimilating ammonia within the *Rhizobium*-containing cell only, thus preventing accumulation of ammonia to toxic concentrations (33, 114).

The conclusion that most amino acid biosynthesis occurs in plastids (83) is commonly cited. As with aromatic amino acids, this conclusion is based mainly upon data showing that most or all enzymes needed for biosynthesis of a given amino acid are present in plastids. Such plastidic location of amino acid biosynthesis would require transport of such amino acids to all other compartments requiring amino acids for protein synthesis or as starting molecules for other biosynthetic pathways. In those cases (83) where sensitive feedback control of enzyme activities is maintained in the plastid (e.g. aspartate-derived and branched-chain amino acids), it is difficult to envision

that the end products would be readily available to other compartments. This is because efficient end-product control implies sensitivity to small pools of end products. In connection with the feedback sensitivity of DS-Mn, CM-1, and AS-1 in plastids and the feedback resistance of DS-Co, CM-2, and AS-2 in the cytoplasm (see Figure 1) it is intriguing that the same differential compartmentation has been found for feedback-sensitive and feedback-resistant isozymes of homoserine dehydrogenase in barley and pea (96).

A number of considerations may explain why extra-plastid pathways may be less easily detected than plastid-localized pathways. Expectations for specific enzyme reactions and characteristics are commonly based upon established prokaryote pathways, especially those of *E. coli*. Since the endosymbiotic hypothesis dictates that chloroplasts and prokaryotes share a common ancestor (76), plastid enzymes are more likely to resemble those of *E. coli* than are cytoplasmic enzymes (63b). When a plastid enzyme is purified, it is sometimes assumed that its antibody would detect isozymes in other compartments. Likewise, where genes have been sequenced, there is a tendency to assume that gene probes would detect other genes (which may not be homologous). The plausible lack of common ancestry (or ancient divergence) of genes specifying isozymes expressed in different subcellular locations could easily result in antibodies that fail to cross-react, and in genes having low percentages of identity. Second, the isolation of plastids is an initial purification step that protects the enzymes from proteases and phenolics released from disrupted vacuoles. Third, as demonstrated for isozymes of glycolysis, the pentose phosphate pathway, and aromatic amino acid biosynthesis, the properties of plastid-localized and cytoplasm-localized isozymes are frequently different to a drastic and opposite extent. Thus, optimization of enzyme assays based upon plastid isozyme characteristics may virtually guarantee lack of ability to detect cytoplasmic isozymes.

PERSPECTIVE

Our understanding of metabolic events in general derives mainly from carbohydrate-, tricarboxylic acid-, and aromatic metabolism. While the first two pathways/cycles are common to all living organisms, aromatic amino acid metabolism is found in microorganisms and plants only. This is also the pathway that has provided some of the most rigorous examples of enzyme-enzyme interactions and channeling of metabolic intermediates, including tunnel effects (52) and long-range interaction of catalytic sites in enzyme complexes (62). The diverse aromatic pathway sections thus seem ideally suited for spatial-localization and pathway-mechanistic studies.

The spatial organization of enzymes in correspondence to the sequence of catalytic steps in a given pathway can exist at many levels. These levels are

not mutually exclusive and include (*a*) physical isolation within compartments (e.g. organelles) or other structures (e.g. cell walls), (*b*) organization of multiple catalytic domains on a single multifunctional protein, and (*c*) the existence of specific protein-protein interactions in the form of dissociable complexes. The breakage of cells in order to prepare enzyme extracts is likely to disrupt and mask even stable forms of organization. Thus, damaged organelles may suffer loss of enzymes by abnormal leakage, multifunctional proteins may be cleaved to monofunctional fragments by exposure to released proteases, and complexes may be dissociated by physical trauma and dilution. Fragile protein-protein interactions may occur at degrees of subtlety that defy easy experimental detection. A continuing and accelerating documentation of the spatial framework of organized enzyme systems that comprise discrete biochemical pathways is clearly inevitable in prokaryote, plant, and animal systems. One can anticipate an eventual appreciation of the spatial organization that underlies the interfacing of particular pathways that link entire metabolic networks.

ACKNOWLEDGMENTS

The research support provided in part by the National Science Foundation DCB 8916265 (to G. H.) and by the United States Department of Energy, Contract DE-FG05-86ER13581 (to R. A. J.) is gratefully acknowledged. The authors thank Dr. W. C. Plaxton for his comments on the manuscript.

Literature Cited

1. Ahmed, S. I., Giles, N. H. 1969. Organization of enzymes in the common aromatic synthetic paythway: evidence for aggregation in fungi. *J. Bacteriol.* 99:231–37
2. Amrhein, N., Deus, B., Gehrke, P., Hollaender, H., Schulz, A., Steinrücken, H. C. 1980. Glyphosate inhibits chorismate formation in vivo and in vitro in buckwheat (*Fagopyrum esculentum* M.). *Plant Physiol.* 65:97–103
3. Anderson, L. E., Duggan, J. X. 1976. Light modulation of glucose 6-phosphate dehydrogenase. Partial characterization of the light inactivation system and its effects on the properties of the chloroplast and cytosolic form of the enzyme. *Plant Physiol.* 58:135–39
4. Aoyagi, K., Bassham, J. A. 1984. Pyruvate orthophosphate dikinase of C_3 seeds and leaves as compared to the enzyme from maize. *Plant Physiol.* 75:387–92
5. Ap Rees, T. 1980. Assessment of the contribution of metabolic pathways to plant respiration. In *The Biochemistry of Plants*. Vol. 2. *Metabolism and Respiration*, ed. D. D. Davis, pp. 1–29. New York: Academic
6. Bagge, P., Larsson, C. 1986. Biosynthesis of aromatic amino acids by highly purified spinach chloroplasts—compartmentation and regulation of the reactions. *Physiol. Plant.* 68:641–47

6a. Batke, J. 1989. Remarks on the supramolecular organization of the glycolytic system in vivo. *FEBS Lett.* 251:13–16

6b. Beeckmans, S., Van Driessche, E., Kanarek, L. 1990. Clustering of sequential enzymes in the glycolytic pathway and the citric acid cycle. *J. Cell Biochem.* 43:297–306

7. Beerhues, L., Robeneck, H., Wiermann, R. 1988. Chalcone synthases from spinach (*Spinacia oleracea* L.). *Planta* 173:544–53
8. Bentley, R. 1990. The shikimate pathway—a metabolic tree with many branches. *Crit. Rev. Biochem. Mol. Biol.* 25:307–84
9. Bickel, H., Palme, L., Schulz, G. 1978. Incorporation of shikimate and other precursors into aromatic amino acids and

prenylquinones of isolated spinach chloroplasts. *Phytochemistry* 17:119–24

9a. Blakeley, S. D., Plaxton, W. C., Dennis, D. T. 1991. Relationship between the subunits of leucoplast pyruvate kinase from *Ricinus communis* and a comparison with the enzyme from other sources. *Plant Physiol.* 96:1283–88

10. Bolwell, G. P., Sap, J., Cramer, C. L., Lamb, C. J., Schuch, W., Dixon, R. A. 1986. L-Phenylalanine ammonia-lyase from *Phaseolus vulgaris:* partial degradation of enzyme subunits in vitro and in vivo. *Biochim. Biophys. Acta* 881:210–21

11. Boudet, A. M., Lecussan, R. 1974. Généralité de l' association (5-déshydroquinate hydro-lyase, shikimate: NAD+ oxydoreductase) chez les végétaux superieurs. *Planta* 119:71–79

11a. Brooks, S. P. J., Storey, K. B. 1991. Where is the glycolytic complex? *FEBS Lett.* 278:135–38

12. Brotherton, J. E., Hauptmann, R. M., Widholm, J. M. 1986. Anthranilate synthase forms in plants and cultured cells of *Nicotiana tabacum* L. *Planta* 168: 214–21

13. Buchanan, B. B. 1980. Role of light in the regulation of chloroplast enzymes. *Annu. Rev. Plant Physiol.* 31:341–74

14. Byng, G. S., Whitaker, R. J., Jensen, R. A. 1985. Glyphosate as a probe of the pentafunctional arom protein of *Euglena gracilis*. *Can. J. Bot.* 63:1021–24

15. Byng, G. S., Whitaker, R. J., Shapiro, C. I., Jensen, R. A. 1981. The aromatic amino acid pathway branches at L-arogenate in *Euglena*. *Mol. Cell Biol.* 1:426–38

16. Charles, J. G., Keyte, J. W., Brammar, W. J., Smith, M., Hawkins, A. R. 1986. The isolation and nucleotide sequence of the complex AROM locus of *Aspergillus nidulans*. *Nucl. Acid Res.* 14:2201–13

17. Clifford, M. N., Kellard, B., Ah-sing, E. 1989. Caffeyltyrosine from green robusta coffee beans. *Phytochemistry* 28:1989–90

18. Coggins, J. R., Boocock, M. R., Chaudhuri, S., Lambert, J. M., Lumsden, J., et al. 1987. The arom multifunctional enzyme from *Neurospora crassa*. *Methods Enzymol.* 142:325–41, 1987

19. Coggins, J. R., Duncan, K., Anton, I. A., Boocock, M. R., Chaudhury, S., et al. 1987. The anatomy of a multifunctional enzyme. *Biochem. Soc. Trans.* 15:754–59

20. Conn, E. E. 1978. Biosynthesis of cyanogenic glycosides. *Naturwissenschaft* 66:28–34

21. Cooper, J. B., Varner, J. E. 1983. Insolubilization of hydroxyproline-rich cell wall glycoprotein in aereated carrot root slices. *Biochem. Biophys. Res. Commun.* 112:161–67

22. Copeland, L., Turner, J. F. 1987. The regulation of glycolysis and the pentose phosphate pathway. In *The Biochemistry of Plants*, ed. D. D. Davis, 11:107–28. New York: Academic

23. Czichi, U., Kindl, H. 1977. Phenylalanine ammonia-lyase and cinnamic acid hydroxylase as assembled consecutive enzymes on microsomal membranes of cucumber cotyledones: cooperation and subcellular distribution. *Planta* 134: 133–43

24. D'Amato, T. A., Ganson, R., Gaines, C. G., Jensen, R. A. 1984. Subcellular localization of chorismate-mutase isoenzymes in protoplasts from mesophyll and suspension-cultured cells of *Nicotiana silvestris*. *Planta* 162:104–8

25. Davis, D. D. 1979. The central role of phosphoenolpyruvate in plant metabolism. *Annu. Rev. Plant Physiol.* 30:131–58

26. Davis, R. H. 1967. Channeling in neurospora metabolism. In *Organizational Biosynthesis*, ed. H. J. Vogel, J. O. Lampen, V. Bryson, pp. 303–33. New York: Academic

27. De-Eknamkul, W., Ellis, B. E. 1987. Tyrosine aminotransferase: the entry-point enzyme of the tyrosine-derived pathway in rosmarinic acid biosynthesis. *Phytochemistry* 26:1941–46

28. DeLeeuw, A. 1967. Gene-enzyme relationships in polyaromatic auxotrophic mutants in *Saccharomyces cerevisiae*. *Genetics* 56:554–55

29. Dennis, D. T., Miernyk, J. A. 1982. Compartmentation of nonphotosynthetic carbohydrate metabolism. *Annu. Rev. Plant Physiol.* 33:27–50

30. Duff, S. M. G., Lefebvre, D. D., Plaxton, W. C. 1989. Purification and characterization of a phosphoenolpyruvate phosphatase from *Brassica nigra* suspension cells. *Plant Physiol.* 90:734–41

31. Duff, S. M. G., Moorhead, G. B. G., Lefebvre, D. D., Plaxton, W. C. 1989. Phosphate starvation inducible "bypasses" of adenylate and phosphate dependent glycolytic enzymes in *Brassica nigra* suspension cells. *Plant Physiol.* 90:1275–78

32. Duncan, K., Edwards, R. M., Coggins, J. R. 1987. The pentafunctional arom enzyme of *Saccharomyces cerevisiae* is

a mosaic of monofunctional domains. *Biochem. J.* 246:375–86
33. Edwards, J. W., Walker, E. L., Coruzzi, G. M. 1990. Cell specific expression in transgenic plants reveals nonoverlaping roles for chloroplast and cytosolic glutamine synthetase. *Proc. Natl. Acad. Sci. USA* 87:3459–63
34. Flügge, U. I., Heldt, H. W. 1984. The phosphate-triose phosphate-phosphoglycerate translocator of the chloroplast. *Trends Biochem. Sci.* 9:530–33
35. Garland, W. J., Dennis, D. T. 1980. Plastid and cytosolic phosphofructokinases from the developing endosperm of *Ricinus communis* L. Separation, purification and initial characterization of the isozymes. *Arch. Biochem. Biophys.* 204:302–9
36. Gasser, C. S., Winter, J. A., Hiranoka, C. M., Shah, D. M. 1988. Structure, expression and avolution of the 5-enolpyruvylshikimate-3-phosphate synthase genes of petunia and tomato. *J. Biol. Chem.* 263:4280–89
37. Gerhardt, R., Heldt, H. W. 1984. Measurement of subcellular metabolite levels in leaves by fractionation of freeze stopped material in nonaqueous media. *Plant Physiol.* 75:542–47
38. Gilchrist, D. G., Kosuge, T. 1980. Aromatic amino acid biosynthesis and its regulation. In *The Biochemistry of Plants*. Vol 5. *Amino Acids and Derivatives*, ed. B. J. Miflin, pp. 507–29. New York: Academic
39. Giles, N. H., Case, M. E., Partridge, C. W. H., Ahmed, S. I. 1967. A gene cluster in *Neurospora crassa* coding for an aggregate of five aromatic synthetic enzymes. *Proc. Natl. Acad. Sci. USA* 58:1453–60
40. Goers, S. K., Jensen, R. A. 1984. Separation and characterization of two chorismate-mutase isoenzymes from *Nicotiana silvestris*. *Planta* 162:109–16
41. Goers, S. K., Jensen, R. A. 1984. The differential allosteric regulation of two chorismate mutase isoenzymes of *Nicotiana silvestris*. *Planta* 162:117–24
42. Gottlieb, L. D. 1982. Conservation and duplication of isozymes in plants. *Science* 216:373–80
43. Hahlbrock, K., Scheel, D. 1989. Physiology and molecular biology of phenylpropanoid metabolism. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40:347–69
44. Hamdan, M. A. M. S., Dixon, R. A. 1987. Differential patterns of protein synthesis in bean cells exposed to elicitor fractions from *Colletotrichum lindemuthianum*. *Physiol. Mol. Plant Pathol.* 31:105–21
45. Hankins, C. N., Mills, S. E. 1976. Anthranilate synthase-amidotransferase (combined). A novel form of anthranilate synthase from *Euglena gracilis*. *J. Biol. Chem.* 251:7774–78
46. Hankins, C. N., Mills, S. E. 1977. A dimer of a single polypeptide chain catalyzes the terminal four reactions of the L-tryptophan pathway in *Euglena gracilis*. *J. Biol. Chem.* 252:235–39
47. Havir, E. A., Hanson, K. R. 1968. L-Phenylalanine ammonia-lyase. II. mechanism and kinetic properties of the enzyme from potato tubers. *Biochemistry* 7:1904–14
48. Hrazdina, G., Jensen, R. A. 1990. Multiple parallel pathways in plant aromatic metabolism? In *Structural and Organizational Aspects of Metabolic Regulation*, ed. P. Srere, M. E. Jones, C. K. Mathews, pp. 27–41. New York: Alan R. Liss
49. Hrazdina, G., Wagner, G. J. 1985. Compartmentation of plant phenolic compounds; sites of synthesis and accumulation. In *Annual Proceedings of the Phytochemical Society of Europe*, ed. C. F. Van Sumere, P. J. Lea, 25:120–33. Oxford: Clarendon Press
50. Hrazdina, G., Wagner, G. J. 1985. Metabolic pathways as enzyme complexes: evidence for the synthesis of phenylpropanoids and flavonoids on membrane associated enzyme complexes. *Arch. Biochem. Biophys.* 237: 88–100
51. Hrazdina, G., Zobel, A. M., Hoch, H. C. 1987. Biochemical, immunological and immunocytochemical evidence for the association of chalcone synthase with endoplasmic reticulum membranes. *Proc. Natl. Acad. Sci. USA* 84:8966–70
52. Hyde, C. C., Ahmed, S. A., Padlan, E. A., Miles, E. W., Davies, D. R. 1988. Three-dimensional structure of the tryptophan synthase $\alpha 2\beta 2$-multienzyme complex from *Salmonella typhimurium*. *J. Biol. Chem.* 263:17857–71
53. Igbavboa, U., Sieweke, H.-J., Leistner, E., Röwer, I., Hüsemann, W., Barz, W. 1985. Alternative formation of anthraquinones and lipoquinones in heterotrophic and photoautotrophic cell suspenson cultures of *Morinda lucida* Benth. *Planta* 166:537–44
54. Ireland, R. J., Deluca, V., Dennis, D. T. 1979. Isoenzymes of pyruvate kinase in etioplasts and chloroplasts. *Plant Physiol.* 63:903–7
55. Ireland, R. J., Dennis, D. T. 1980. Isozymes of the glycolytic and pentose

phosphate pathways in storage tissues of different oil seeds. *Planta* 149:476–79
56. Jensen, R. A. 1986. The shikimate/arogenate pathway: link between carbohydrate metabolism and secondary metabolism. *Physiol. Plant.* 66:164–68
57. Jensen, R. A. 1986. Tyrosine and phenylalanine biosynthesis: relationship between alternative pathways, regulation and subcellular location. *Rec. Adv. Phytochem.* 20:57–82
58. Jensen, R. A., Morris, P., Bonner, C., Zamir, L. O. 1989. Biochemical interface between aromatic amino acid biosynthesis and secondary metabolism. In *Biosynthesis and Biodegradation of Plant Cell Wall Polymers*, ed. N. G. Lewis, M. G. Paice, 399:89–107. New York: Am. Chem. Soc. Symp. Ser.
59. Johnson, H. S. 1972. Dithiothreitol: an inhibitor of glucose 6-phosphate dehydrogenase activity in leaf extracts and isolated chloroplasts. *Planta* 106:273–77
59a. Keleti, T., Ovádi, J. 1988. Control of metabolism by dynamic macromolecular interactions. *Curr. Top. Cell Reg.* 29:1–33
60. Kelly, G. J., Latzko, E. 1977. Chloroplast phosphofructokinase I. Proof of phosphofructokinase activity in chloroplasts. *Plant Physiol.* 60:290–94
61. Kelly, G. J., Latzko, E. 1977. Chloroplast phosphofructokinase II. Partial purification and regulatory properties. *Plant Physiol.* 60:295–99
62. Kirschner, K., Lane, A. N., Strasser, A. W. M. 1991. Reciprocal communication between the lyase and synthase active sites of the tryptophan synthase bienzyme complex. *Biochemistry* 30:472–78
63. Klee, H. J., Muskopf, Y. M., Gasser, C. S. 1987. Cloning of an *Arabidopsis thaliana* gene encoding 5-enolpyruvylshikimate-3-phosphate synthase: sequence analysis and manipulation to obtain glyphosate-tolerant plants. *Mol. Gen. Genet.* 210:437–42
63a. Knowles, V. L., Greison, M. F., Dennis, D. T. 1990. Characterization of ATP-dependent fructose 6-phosphate 1-phosphotransferase isozymes from leaf and endosperm tissues of *Ricinus communis*. *Plant Physiol.* 92:155–59
63b. Knowles, V. L., Dennis, D. T., Plaxton, W. C. 1989. Purification of a novel pyruvate kinase from a green alga. *FEBS Lett* 259:130–32
64. Koshiba, T. 1978. Purification of the two forms of the associated 3-dehydroquinate hydro-lyase and shikimate: $NADP^+$ oxidoreductase in *Phaseolus mungo* seedlings. *Biochem. Biophys. Acta* 522:10–18
65. Lamport, D. T. A. 1965. The protein component of primary cell walls. *Adv. Bot. Res.* 2:151–218
66. Larsen, P. O., Wieczorkowska, E. 1978. Transformation of 3-(3-carboxylphenyl)alanine in *Iris* species. *Biochim. Biophys. Acta* 542:253–62
67. Larson, P. O., Onderka, D. K., Floss, H. G. 1972. Biosynthesis of phenylalanine, tyrosine, 3-(3-carboxyphenyl)alanine and 3-(3-carboxy-4-hydroxyphenyl)alanine in higher plants. Examples of the transformation possibilities for chorismic acid. *Biochim. Biophys. Acta* 381:397–408
68. Lea, P. J., Joy, K. W. 1983. Amino acid conversion in germinating seeds. *Recent Adv. Phytochem.* 17:77–109
69. Leete, E. 1979. Biosynthesis and metabolism of tropane alkaloids. *Planta Med.* 36:97–112
70. Lehninger, A. L. 1991. *Biochemistry*, pp. 417–41. New York: Worth Publishers
71. Lendzian, K. J. 1978. Interactions between Mg-ions, pH, glucose 6-phosphate and NADPH/$NADP^+$ ratios in the modulation of chloroplast glucose 6-phosphate dehydrogenase in vitro. *Planta* 141:105–10
72. Liedvogel, B. 1986. Acetyl Coenzyme A and isopentenyl pyrophosphate as lipid precursors in plant cells-biosynthesis and compartmentation. *J. Plant Physiol.* 124:211–22
73. Luckner, M. 1977. *Secondary Metabolism in Microorganisms, Plants and Animals*, pp. 32–45. Berlin:Springer-Verlag
74. MacNab, R., Moses, V., Mowbray, J. 1973. Evidence for metabolic compartmentation in *Escherichia coli*. *Eur. J. Biochem.* 34:15–19
75. Margna, U., Vainjarv, T. 1981. Buckwheat seedling flavonoids do not undergo rapid turnover. *Biochem. Physiol. Pflanz.* 176:44–53
76. Margulis, L. 1970. *Origin of eukaryotic cells*. New Haven: Yale Univ. Press
77. Marques, I. A., Brodelius, P. E. 1988. Elicitor-induced L-tyrosine decarboxylase from plant cell suspension cultures. *Plant Physiol.* 88:52–55
78. Masters, C. J., Reid, S., Don, M. 1987. Glycolysis—new concepts in an old pathway. *Mol. Cell. Biochem.* 76:3–14
79. Mavandad, M., Edwards, R., Liang, X., Lamb, C. J., Dixon, R. A. 1990. Effects of *trans*-cinnamic acid on expression of the bean phenylalanine

ammonia-lyase gene family. *Plant Physiol.* 94:671–80
80. McFarlane, I. J., Lees, E. M., Conn, E. E. 1975. The in vitro biosynthesis of dhurrin, the cyanogenic glycoside of *Sorghum* bicolor. *J. Biol. Chem.* 250: 4708–13
81. Mellon, J. E., Helgeson, J. P. 1982. Interaction of a hydroxyproline-rich glycoprotein from tobacco callus with potential pathogens. *Plant Physiol.* 70: 401–5
82. Miernyk, J. A., Camp, P. J., Randall, D. D. 1985. Regulation of plant pyruvate dehydrogenase complexes. *Curr. Top. Plant Biochem. Physiol.* 4:175–90
83. Miflin, B. J., Lea, P. J. 1982. Ammonia assimilation and amino acid metabolism. In *Encyclopedia of Plant Physiology, New Ser.*, ed. D. Boulter, B. Parthier, 14A:5–64. Berlin: Springer-Verlag
83a. Moorhead, G. B. G., Plaxton, W. C. 1988. Binding of glycolytic enzymes to a particulate fraction in carrot and sugar beet storage roots. *Plant Physiol.* 86: 348–51
84. Morris, P. F., Bonner, C. A., Doong, R. L., Subramanian, P., Fischer, R., Jensen, R. A. 1989. Mechanical wounding induces the entire primary pathway of aromatic amino acid biosynthesis. *Plant Physiol. (Life Sci. Adv.) (Ser. B)* 8: 35–39
85. Morris, P. F., Doong, R. L., Jensen, R. A. 1989. Evidence from *Solanum tuberosum* in support of the dual-pathway hypothesis of aromatic biosynthesis. *Plant Physiol.* 89:10–14
86. Mousdale, D. M., Campbell, M. S., Coggins, J. R. 1987. Purification and characterization of bifunctional dehydroquinase-shikimate: NADP oxidoreductase from pea seedlings. *Phytochemistry* 26:2665–70
87. Mousdale, D. M., Coggins, J. R. 1985. Subcellular localization of the common shikimate-pathway enzymes in *Pisum sativum* L. *Planta* 163:241–49
88. Oliver, D. J., Neuburger, M., Bourguignon, J., Douce, R. 1990. Interaction between the component enzymes of the glycine decarboxylase multienzyme complex. *Plant Physiol.* 94:833–39
89. Ottaway, J. H., Mowbray, J. 1977. The role of compartmentation in the control of glycolysis. In *Current Topics in Cellular Regulation*, ed. B. L. Horecker, E. R. Stadtman, pp. 107–208. New York: Academic
90. Patel, V. B., Giles, N. H. 1979. Purification of the arom multienzyme aggregate from *Euglena gracilis*. *Biochim. Biophys. Acta* 567:24–34
90a. Ovádi, J. 1988. Old pathway—new concept: control of glycolysis by metabolite-modulated dynamic enzyme associations. *TIBS* 13:486–90
91. Pennings, J. M., Groen, B. W., Duine, J. A., Verpoorte, R. 1989. Tryptophan decarboxylase from *Cathranthus roseus* is a pyridoxo-quinoprotein. *FEBS Lett.* 255:97–100
91a. Plaxton, W. C. 1990. Biochemical regulation. In *Plant Physiology, Biochemistry, and Molecular Biology*, ed. D. T. Dennis, D. H. Turpin, pp. 29–44. London: Longman
91b. Plaxton, W. C., Dennis, D. T., Knowles, V. L. 1990. Purification of leucoplast pyruvate kinase from developing castor bean endosperm. *Plant Physiol.* 94:1528–34
91c. Plaxton, W. C. 1989. Molecular and immunological characterization of plastid and cytosolic pyruvate kinase isozymes from castor-oil plant endosperm and leaf. *Eur. J. Biochem.* 181:443–51
92. Plaxton, W. C., Storey, K. B. 1986. Glycolytic enzyme binding and metabolic control in anaerobiosis. *J. Comp. Physiol.* 156:635–40
93. Poulsen, C., Verpoorte, R. 1991. Roles of chorismate mutase, isochorismate synthase and anthranilate synthase in plants. *Phytochemistry* 30:377–86
94. Ream, J. L., Steinrücken, M. C., Porter, C. A., Sikorski, J. A. 1988. Purification and properties of 5-enolpyruvylshikimate-3-phosphate synthase from dark grown seedlings of *Sorghum bicolor*. *Plant Physiol.* 82:230–38
95. Reed, L. J., Cox, D. J. 1970. Multienzyme complexes. In *The Enzymes*, ed. P. D. Boyer, pp. 213–40. New York: Academic
96. Sainis, J. K., Mayne, R. G., Wallsgrove, R. M., Lea, P. J., Miflin, B. J. 1981. Localization and characterization of homoserine dehydrogenase isolated from barley and pea leaves. *Planta* 152:491–96
97. Sandberg, G., Gardeström, P., Sitbon, F., Olsson, O. 1990. Presence of indole-3-acetic acid in chlorplasts of *Nicotiana tabacum* and *Pinus sylvestris*. *Planta* 180:562–68
98. Schnarrenberger, C., Oeser, A., Tolbert, N. A. 1973. Two isozymes each of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in spinach leaves. *Arch. Biochem. Biophys.* 154:438–48
99. Shimada, M., Conn, E. E. 1977. The enzymatic conversion of p-hydroxyphenylacetaldoxime to p-hydroxymandelonitrile. *Arch. Biochem. Biophys.* 180:199–207

100. Simantiras, M., Leistner, E. 1989. Formation of *o*-succinylbenzoic acid from iso-chorismic acid in protein extracts from anthraquinone-producing plant cell suspension cultures. *Phytochemistry* 28:1381–82
101. Singh, B. K., Connelly, J. A., Conn, E. E. 1985. Chorismate mutase isozymes from *Sorghum bicolor:* purification and properties. *Arch. Biochem. Biophys.* 243:374–84
102. Smart, C. C., Amrhein, N. 1987. Ultrastructural localization by protein A-gold immunocytochemistry of 5-enolpyruvylshikimic acid 3-phosphate synthase in a plant cell culture which overproduces the enzyme. *Planta* 170:1–6
102a. Srere, P. A. 1987. Complexes of sequential metabolic enzymes. *Annu. Rev. Biochem.* 56:21–56
103. Srere, P. A., Mosbach, K. 1974. Metabolic compartmentation: symbiotic, organellar, multienzymic, and microenvironmental. *Annu. Rev. Microbiol.* 28:61–83
104. Srere, P. A., Sümegi, B., Sherry, A. D. 1987. Organizational aspects of the citric acid cycle. *Biochem. Soc. Symp.* 54:173–82
104a. Srere, P. A. 1990. Citric acid cycle redux. *TIBS* 15:411–12
105. Srivastava, D. K., Bernhard, S. A. 1986. Metabolite transfer via enzyme-enzyme complexes. *Science* 234:1081–84
106. Stafford, H. A. 1974. Possible multienzyme complexes regulating the formation of C_6—C_3 phenolic compounds and lignins in higher plants. *Rec. Adv. Phytochem.* 8:53–79
107. Stafford, H. A. 1981. Compartmentation in natural product biosynthesis by multienzyme complexes. In *The Biochemistry of Plants,* ed. E. E. Conn, pp. 118–37. New York: Academic
108. Sung, S. S., Xu, D., Galloway, C. M., Black, C. C. 1987. A reassessment of glycolysis and gluconeogenesis in higher plants. *Physiol. Plant.* 72:650–54
109. Tingey, S. V., Tsai, F. Y., Edwards, J. W., Walker, E. L., Coruzzi, G. M. 1988. Chloroplast and cytosolic glutamine synthetase are encoded by homologous nuclear genes which are differentialy expressed in vivo. *J. Biol. Chem.* 263:9651–57
110. Ureta, T. 1978. The role of isozymes in metabolism: a model of metabolic pathways as the basis for the biological role of isozymes. *Curr. Top. Cell. Regul.* 13:233–58
111. Urquhart, A. A., Joy, K. W. 1981. Use of phloem exudate technique in the study of amino acid transport in pea seeds. *Plant Physiol.* 68:750–54
112. Vértessy, B., Ovádi, J. 1987. A simple approach to detect active-site directed enzyme-enzyme interactions. The aldolase/glycerol phosphate dehydrogenase enzyme system. *Eur. J. Biochem.* 86: 655–59
113. Wagner, G. J., Hrazdina, G. 1984. Endoplasmic reticulum as a site of phenylpropanoid and flavonoid metabolism in *Hippeastrum. Plant Physiol.* 74:901–6
114. Walker, E. L., Coruzzi, G. M. 1989. Developmentally regulated expression of the gene family for cytosolic glutamine synthetase in *Pisum sativum. Plant Physiol.* 91:702–8
115. Walker, J. L., Oliver, D. J. 1986. Glycine decarboxylase multienzyme complex, purification, and partial characterization from pea leaf mitochondria. *J. Biol. Chem.* 261:2214–21
116. Walter, M. H., Hahlbrock, K. 1984. Cinnamic acid induces PAL mRNA and inhibits mRNA translation in cultured parsley cells. *Plant Physiol.* 75:155–56
117. Welch, G. R., Gaertner, F. H. 1975. Influence of an aggregated multienzyme system on transient time: kinetic evidence for compartmentation by an aromatic-amino-acid-synthesizing complex of *Neurospora crassa. Proc. Natl. Acad. Sci. USA* 72:4218–22
118. Welch, G. R., Gaertner, F. H. 1976. Coordinate activation of a multienzyme complex by the first substrate. Evidence for a novel regulatory mechanism in the polyaromatic pathway of *Neurospora crassa. Arch. Biochem. Biophys.* 172: 476–89
119. Wildner, G. F. 1975. The regulation of glucose 6-phosphate in chloroplasts. *Z. Naturforsch.* 30c:756–60
120. Wiskich, J. T., Dry, I. B. 1985. The tricarboxylic acid cycle in plant mitochondria: its operation and regulation. In *Higher Plant Respiration. Encyclopedia of Plant Physiology, New Ser.,* ed. R. Douce, D. A. Day, 13:281–313. Berlin: Springer
121. Wiskich, J. T. 1980. Control of the Krebs cycle. In *The Biochemistry of Plants,* ed. D. D. Davis, 2:243–78. New York: Academic
121a. Wu, X., Gutfreund, H., Lakatos, S., Chock, P. B. 1991. Substrate channeling in glycolysis: a phantom phenomenon. *Proc. Natl. Acad. Sci. USA* 88:497–501
122. Zamir, L. O., Devor, K. A., Jensen, R. A., Tiberio, R., Sauriol, F., Mamer, O. 1991. Biosynthesis of isochorismate in *Klebsiella pneumoniae:* origin of O-2. *Can. J. Microbiol.* 37:276–80

Annual Review of Plant Physiol. Plant Mol. Biol.
Volume 43 (1992)

CONTENTS