BIOSYNTHESIS OF AMINO ACIDS

M.Sc. Biochemistry
1st Yr/2nd Sem

(Note: This E-Content has been prepared as an exclusive reading material for students without any commercial interest. Original contributors are gratefully acknowledged.)

NAME OF TEACHER	**ROLEE SHARMA**
MOBILE NUMBER	**9336576545**
EMAIL ID	**roleesharma@csjmu.ac.in, roleesh@gmail.com**
DESIGNATION	**Professor**
UNIVERSITY NAME	**C.S.J.M. University, Kanpur**
COLLEGE NAME	**C.S.J.M. University, Kanpur**
STREAM NAME	**BIOLOGY**
FACULTY NAME	**SCIENCE**
DEPARTMENT NAME	**BIOSCIENCES**
SUBJECT NAME	**CELL BIOLOGY**
PROGRAM	**M.Sc. BIOCHEMISTRY**
COURSE NAME	**Bioenergetics & Intermediary Metabolism**
PROGRAM DURATION	**2 Years**
SUBTOPIC NAME	**Biosynthesis of amino acids & regulation**
CONTENT TYPE	**PDF**
SEARCH KEYWORD	**Biosynthesis of amino acids**

Amino Acid Biosynthesis

Objectives:

To acquaint the students about:

i) Understanding the biosynthesis of amino acids by different pathways and their regulation

Prelude:

Understanding the mechanism of Nitrate assimilation

Understanding the mechanism of Ammonium assimilation

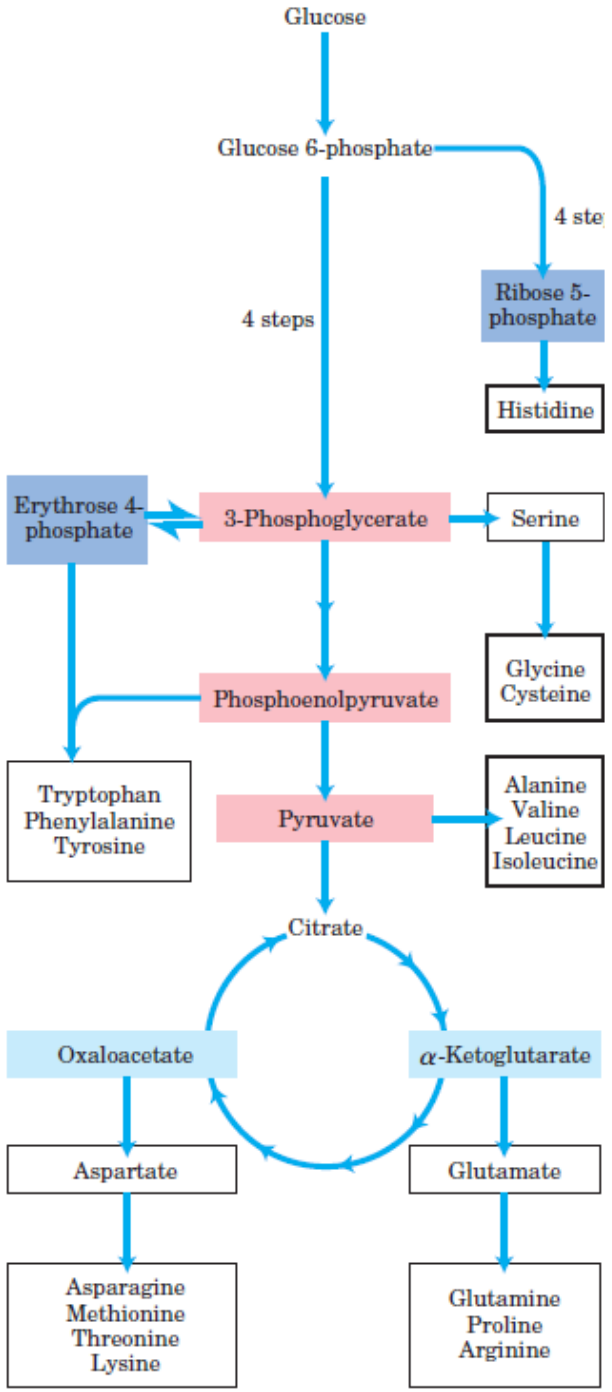
Understanding the Alternative pathways for conversion of ammonium to amino acids

Knowing the transport forms of nitrogen in plant tissues.

Biosynthesis of Amino Acids

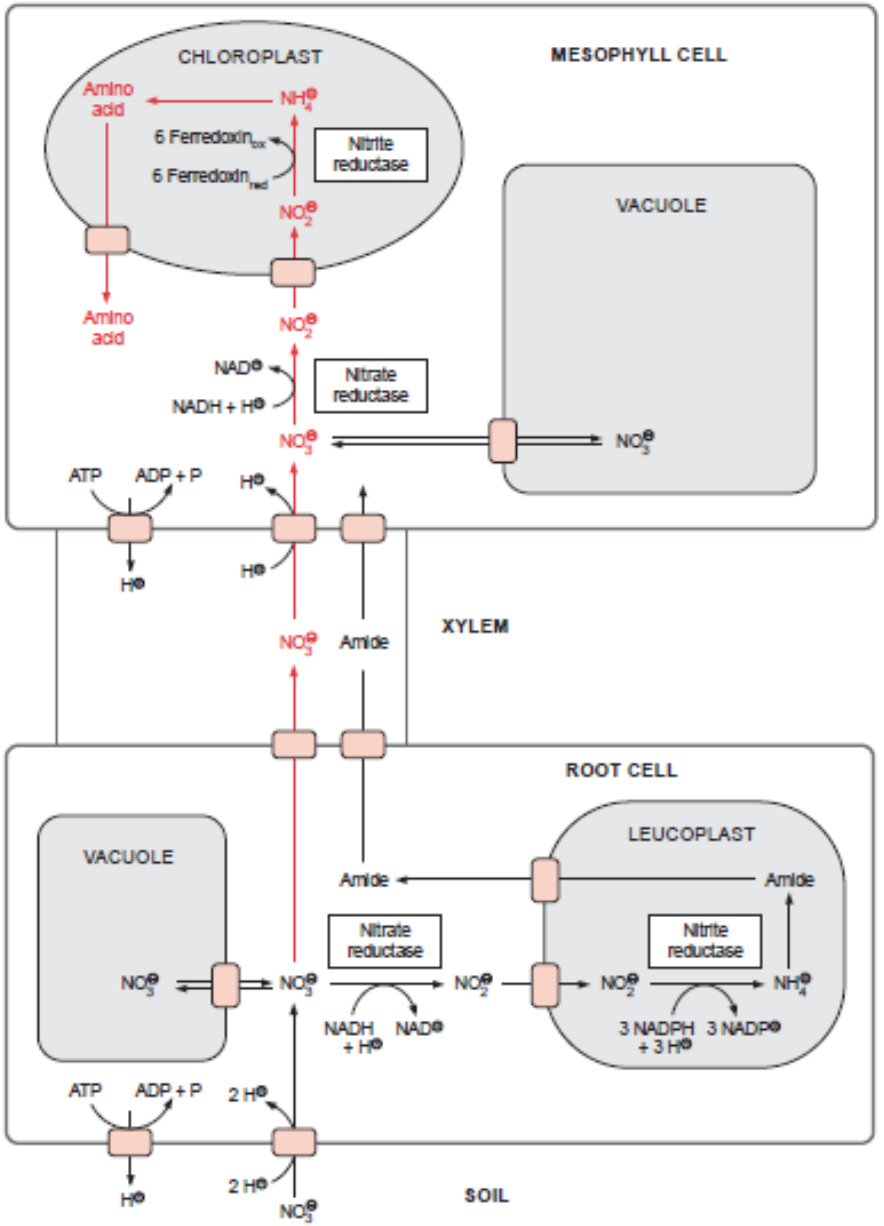
All amino acids are derived from intermediates in glycolysis, the citric acid cycle, or the pentose phosphate pathway. Nitrogen enters these pathways by way of glutamate and glutamine. Some pathways are simple, others are not. Ten of the amino acids are just one or several steps removed from the common metabolite from which they are derived. The biosynthetic pathways for others, such as the aromatic amino acids, are more complex.

Organisms vary greatly in their ability to synthesize the 20 common amino acids. Whereas most bacteria and plants can synthesize all 20, mammals can synthesize only about half of them—generally those with simple pathways. These are the **nonessential amino acids,** not needed in the diet (see Table 18–1). The remainder, the **essential amino acids,** must be obtained from food. Unless otherwise indicated, the pathways for the 20 common amino acids presented below are those operative in bacteria.



Overview of amino acid biosynthesis. The carbon skeleton precursors derive from three sources: glycolysis (pink), the citric acid cycle (blue), and the pentose phosphate pathway (purple).

PRELUDE



The reduction of nitrate to NH_3 proceeds in two partial reactions

Nitrate is assimilated in leaves (In most herbs) and also in the roots (mainly in woody plants and legumes). The transport of nitrate into the root cells proceeds as symport with two protons (Fig). A proton gradient across the PM, generated by a H+-P-ATPase drives the uptake of nitrate against a concentration gradient. The ATP required for formation of proton gradient is mostly provided by mitochondrial respiration. When inhibitors or uncouplers of respiration abolish mitochondrial ATP synthesis in roots, nitrate uptake normally comes to a stop. Root cells contain several nitrate transporters in their PM; including those with a relatively low affinity (half saturation >500 mM nitrate) and those with a v. high affinity (half saturation 20–100 mM nitrate), where the latter is induced only when required by metabolism. In this way the capacity of nitrate uptake into the roots is adjusted to environmental conditions. The efficiency of the nitrate uptake systems permits plant growth when the external nitrate concentration is as low as 10 mM. The nitrate taken up into root cells can be stored there temporarily in the vacuole. Nitrate is reduced to NH4+ in epidermal and cortical cells of the root. This NH4+ is mainly used for the synthesis of glutamine and asparagine (collectively named amide in Fig). These two amino acids can be transported to leaves via xylem vessels.

Nitrate assimilation in roots and leaves of a plant. Nitrate is taken up from soil by root. It can be stored in vacuoles of root cells or assimilated in cells of root epidermis and cortex. Surplus nitrate is carried via xylem vessels to mesophyll cells, where nitrate can be temporarily stored in vacuole. Nitrate is reduced to nitrite in cytosol and then nitrite is reduced further in chloroplasts to NH4+, from which amino acids are formed. H+ transport out of cells of the root and the mesophyll proceeds via a H+-P-ATPase.

Nitrate is reduced to nitrite in the cytosol

Nitrate reduction uses mostly NADH as reductant, although some plants contain a nitrate reductase reacting with NADPH as well as with NADH.

The reduction of nitrite to ammonia proceeds in the plastids

The reduction of nitrite to ammonia requires the uptake of six electrons. This reaction is catalyzed by only one enzyme, the nitrite reductase (Fig. 10.4), which is located exclusively in plastids. This enzyme utilizes reduced ferredoxin as electron donor, which is supplied by photosystem I as a product of photosynthetic electron transport (Fig. 3.31). To a much lesser extent, the ferredoxin required for nitrite reduction in a leaf can also be provided during darkness via reduction by NADPH, which is generated by the oxidative pentose phosphate pathway present in chloroplasts and leucoplasts (Figs. 6.21 and 10.8).

The fixation of NH_4^+ proceeds in the same way as in photorespiration

Glutamine synthetase in the chloroplasts transfers the newly formed NH_4^+ at the expense of ATP to glutamate, forming glutamine (Fig. 10.6). The activity of glutamine synthetase and its affinity for NH_4^+ (*K*m 5 ◊ 10^{-6} mol/L) are so high that the NH_4^+ produced by nitrite reductase is taken up completely.

The same reaction also fixes the NH_4^+ released during photorespiration. Because of the high rate of photorespiration, the amount of NH_4^+ produced by the oxidation of glycine is about 5 to 10 times higher than the amount of NH_4^+ generated by nitrate assimilation. Thus only a minor proportion of glutamine synthesis in the leaves is actually involved in nitrate assimilation. Leaves also contain an isoenzyme of glutamine synthetase in their cytosol.

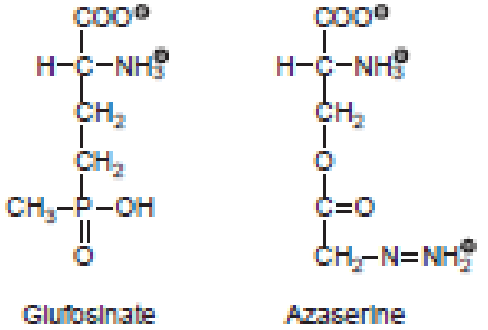
Glufosinate, a substrate analogue of glutamate, inhibits glutamine synthesis. Plants in which the addition of glufosinate has inhibited the synthesis of glutamine accumulate toxic levels of ammonia and die off.

NH4+-glufosinate is distributed as an herbicide under the trade name Liberty (Aventis). It has the advantage that it is degraded rapidly in the soil, leaving behind no toxic degradation products. Recently glufosinate resistant crop plants have been generated by genetic engineering, enabling

the use of glufosinate as a selective herbicide for eliminating weeds in growing cultures.

The glutamine formed in the chloroplasts is converted via glutamate synthase (also called glutamine-oxoglutarate amino transferase, abbreviated **GOGAT**), by reaction with a-ketoglutarate to two molecules of glutamate with ferredoxin as reductant. Some chloroplasts and leucoplasts also contain an NADPH-dependent glutamate synthase. Glutamate synthases are inhibited by the substrate analogue **azaserin**e, which is toxic to plants.

a-Ketoglutarate, which is required for the glutamate synthase reaction, is transported into the chloroplasts by a specific translocator in counter exchange for malate, and the glutamate formed is transported out of the chloroplasts into the cytosol by another translocator, also in exchange for malate (Fig.). A further translocator in the chloroplast envelope transports glutamine in counter-exchange for glutamate, enabling the export of glutamine from the chloroplasts.



Glufosinate (also called phosphinotricin) is a substrate analogue of glutamate and a strong inhibitor of glutamine synthetase. Ammonium glufosinate is an herbicide (Liberty, Aventis).

Azaserine is a substrate analogue of glutamate and an inhibitor of glutamate synthase.

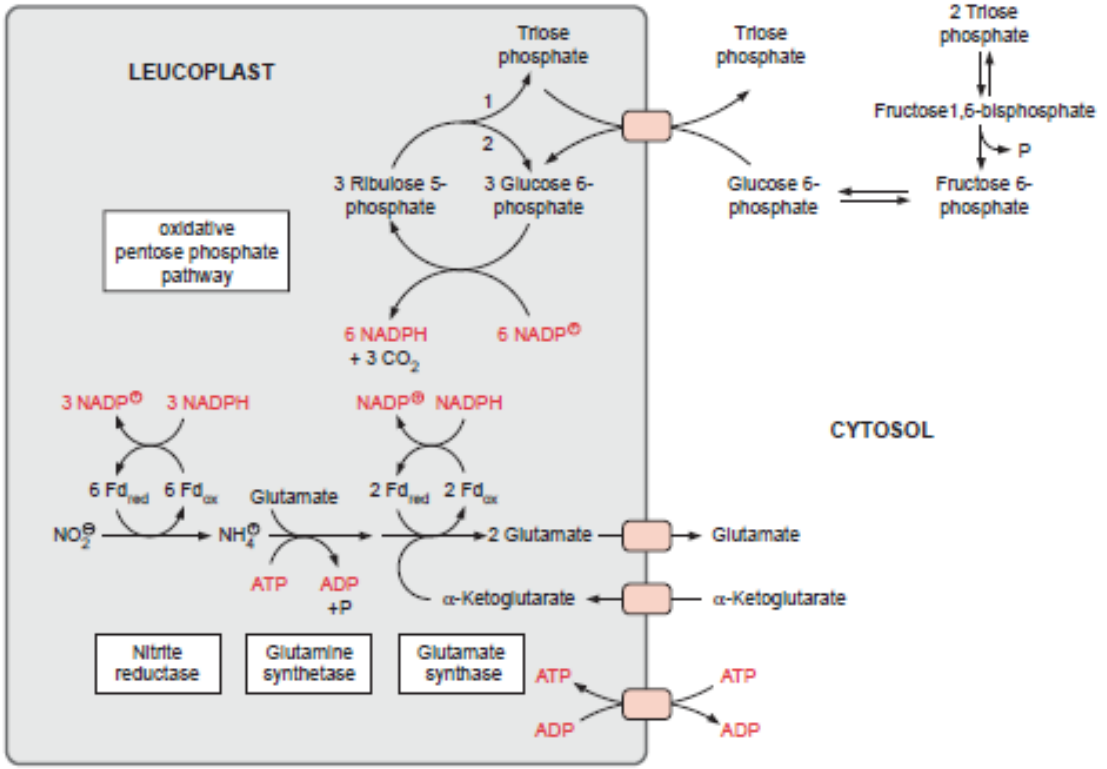
Nitrate assimilation also takes place in the roots

Nitrate assimilation occurs in part, and in some spps. even mainly, in the roots. NH4+ taken up from soil is normally fixed in roots. The reduction of nitrate and nitrite as well as NH4+ fixation proceeds in root cells in an analogous way to the mesophyll cells. However, in the root cells the necessary reducing equivalents are supplied exclusively by oxidation of carbohydrates. The reduction of nitrite and the subsequent fixation of NH4+ occur in the leucoplasts, a differentiated form of plastids.

The oxidative pentose phosphate pathway provides reducing equivalents for nitrite reduction in leucoplasts

The reducing equivalents required for the reduction of nitrite and the formation of glutamate are provided in leucoplasts by oxidation of glucose 6-phosphate via the oxidative PP pathway.

The uptake of glucose 6-phosphate proceeds in counterexchange for triose phosphate. The **glucose 6-phosphate-phosphate translocator** of leucoplasts differs from the triose phosphate-phosphate translocator of chloroplasts in transporting glucose 6-phosphate in addition to phosphate, triose phosphate, and 3-PGA. In the oxidative PP pathway, 3 molecules of glucose 6-phosphate are converted to 3 molecules of ribulose 5-phosphate with the release of 3 molecules of CO2, yielding 6 molecules of NADPH. The subsequent reactions yield 1 molecule of triose phosphate and 2 molecules of fructose 6-phosphate; the latter are reconverted to glucose 6-phosphate via hexose phosphate isomerase. In the cytosol, glucose 6-phosphate is regenerated from 2 molecules of triose phosphate via aldolase, cytosolic fructose 1,6-bisphosphatase, and hexose phosphate isomerase. In this way glucose 6-phosphate can be completely oxidized to CO2 in order to produce NADPH.



The oxidative PPP provides reducing equivalents for nitrite reduction in plastids (leucoplasts) from non-green tissues In some plastids, glucose 1-phosphate is transported in counter-exchange for triose phosphate or phosphate.

As in chloroplasts, nitrite reduction in leucoplasts also requires reduced ferredoxin as reductant. In the leucoplasts, ferredoxin is reduced by NADPH, which is generated by the oxidative pentose phosphate pathway.
The ATP required for glutamine synthesis in the leucoplasts can be generated by the mitochondria and transported into the leucoplasts by a plastid ATP translocator in counter-exchange for ADP. Also, the glutamate synthase of the leucoplasts uses reduced ferredoxin as redox partner, although some leucoplasts also contain a glutamate synthase that utilizes NADPH or NADH directly as reductant. Nitrate reduction in the roots provides the shoot with organic nitrogen compounds mostly as **glutamine** and **asparagine** via the transpiration stream in the xylem vessels. This is also the case when NH4+ is the nitrogen source in the soil.

The end product of nitrate assimilation is a whole spectrum of amino acids

As described earlier, the carbohydrates formed as the product of CO_2 assimilation are transported from the leaves via the sieve tubes to various parts of the plants only in defined transport forms, such as sucrose, sugar alcohols (e.g., sorbitol), or raffinoses, depending on the species.
There are no such special transport forms for the products of nitrate assimilation. All amino acids present in the mesophyll cells are exported via the sieve tubes. Therefore the sum of amino acids can be regarded as the final product of nitrate assimilation. Synthesis of these amino acids takes place mainly in the chloroplasts. The pattern of the amino acids synthesized varies largely, depending on the species and the metabolic conditions. ***In most cases glutamate and glutamine represent the major portion of the synthesized amino acids.***
Glutamate is exported from the chloroplasts in exchange for malate and glutamine in exchange for glutamate (Fig. 10.6). Also, serine and glycine, which are formed as intermediate products in the photorespiratory cycle, represent a considerable portion of the total amino acids present in the mesophyll cells. Large amounts of alanine are often formed in C4 plants.

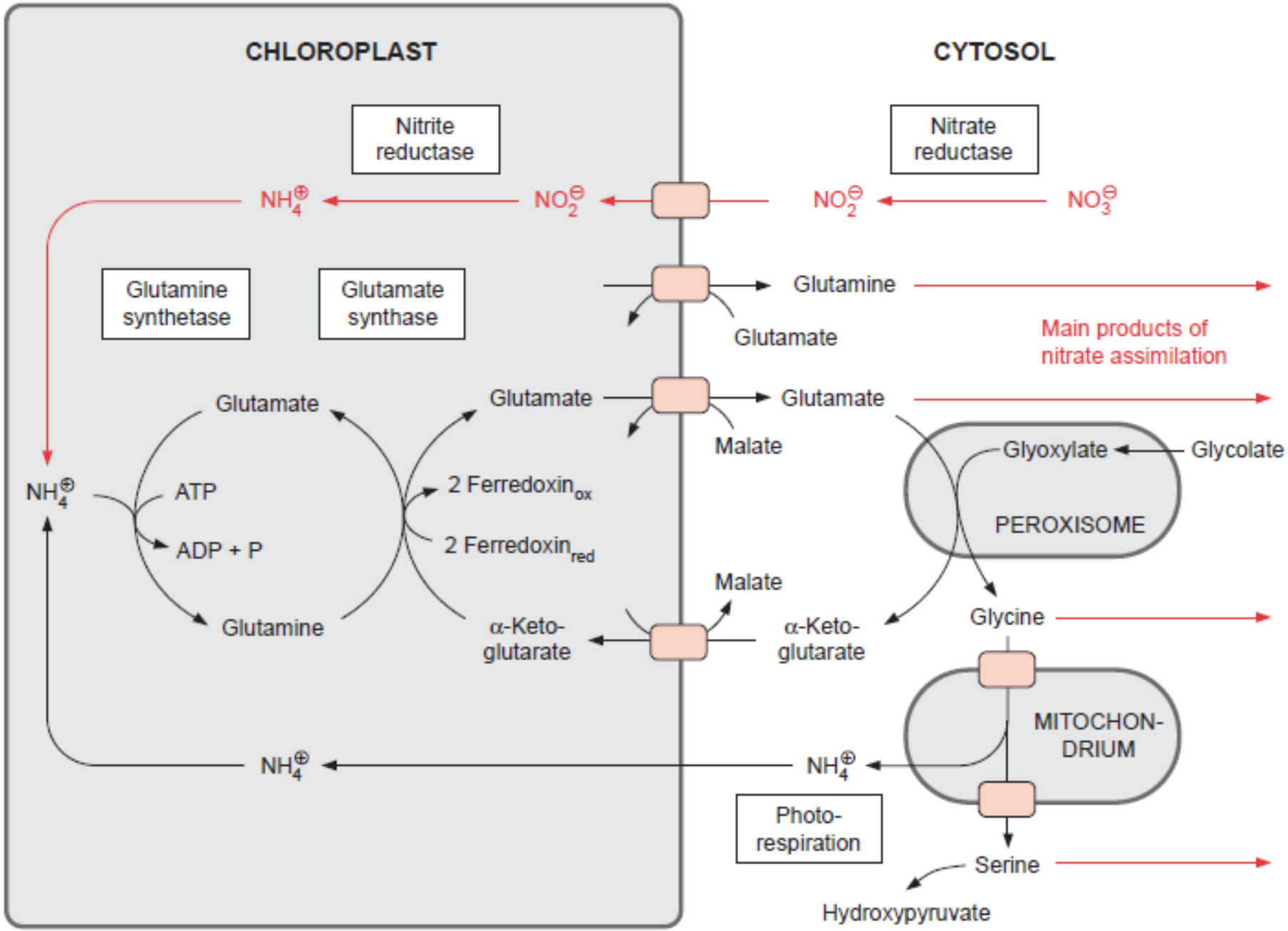
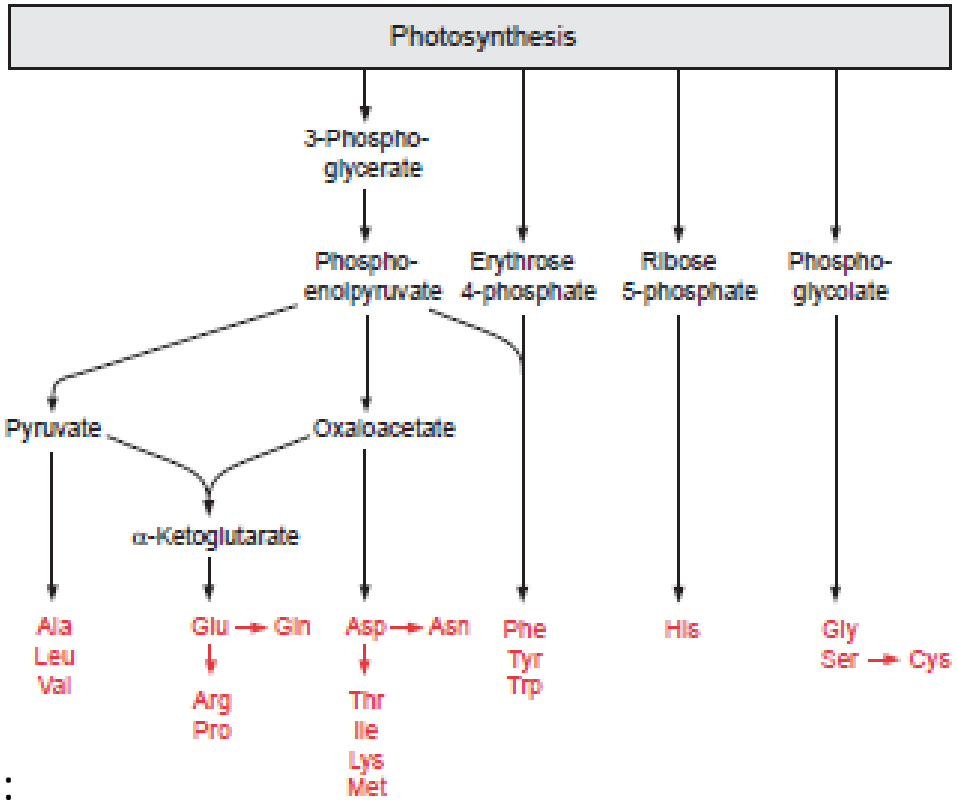


Fig 10.6 Compartmentation of partial reactions of nitrate assimilation and photorespiratory pathway in mesophyll cells. NH_4^+ formed in photorespiratory pathway is colored black and NH_4^+ formed by nitrate assimilation is colored red. The main products of nitrate assimilation are marked with a red arrow.

BIOSYNTHESIS OF AMINO ACIDS

CO_2 assimilation provides the carbon skeletons required for the synthesis of the various amino acids



3-Phosphoglycerate is the most important carbon precursor for the synthesis of amino acids. It is generated in the Calvin cycle and is exported from the chloroplasts to the cytosol by the triose phosphate-phosphate translocator in exchange for phosphate (Fig. 10.11). 3-Phosphoglycerate is converted in the cytosol by phosphoglycerate mutase and enolase to phosphoenolpyruvate (PEP). From PEP two pathways branch off, the reaction via pyruvate kinase leading to pyruvate, and via PEP-carboxylase to oxaloacetate.

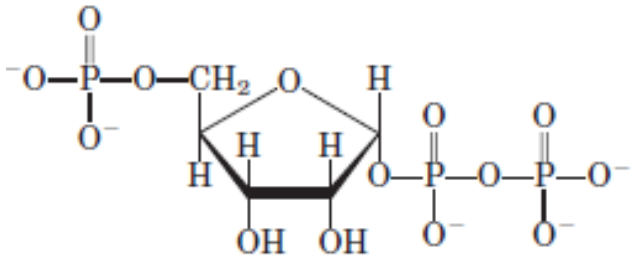
Moreover, PEP together with erythrose 4-phosphate is the precursor for the synthesis of aromatic amino acids via the shikimate pathway, discussed later in this chapter. Since the shikimate pathway is located in the chloroplasts, the required PEP is transported via a specific PEP-phosphate translocator into the chloroplasts.

Oxaloacetate formed by PEP-carboxylase has two functions in nitrate assimilation:

1. It is converted by transamination to aspartate, which is the precursor for the synthesis of five other amino acids (asparagine, threonine, isoleucine, lysine, and methionine).
2. Together with pyruvate it is the precursor for the formation of α-ketoglutarate, which is converted by transamination to glutamate, being the precursor of three other amino acids (glutamine, arginine, and proline).

Glycolate formed by photorespiration is the precursor for the formation of glycine and serine, and from the latter cysteine is formed. In non-green cells, serine and glycine can also be formed from 3-phosphoglycerate. Ribose 5-phosphate is the precursor for the synthesis of histidine. This pathway has not yet been fully resolved in plants.

A useful way to organize these biosynthetic pathways is to group them into six families corresponding to their metabolic precursors (Table 22–1). In addition to these six precursors, there is a notable intermediate in several pathways of amino acid and nucleotide synthesis—5-phosphoribosyl-1-pyrophosphate (PRPP):



PRPP is synthesized from ribose 5-phosphate derived from the pentose phosphate pathway, in a reaction catalyzed by ribose phosphate pyrophosphokinase:

Ribose 5-phosphate + ATP ⟶
5-phosphoribosyl-1-pyrophosphate + AMP

This enzyme is allosterically regulated by many of the biomolecules for which PRPP is a precursor.

TABLE 22–1 Amino Acid Biosynthetic Families, Grouped by Metabolic Precursor

α-Ketoglutarate
Glutamate
Glutamine
Proline
Arginine

3-Phosphoglycerate
Serine
Glycine
Cysteine

Oxaloacetate
Aspartate
Asparagine
Methionine*
Threonine*
Lysine*

Pyruvate
Alanine
Valine*
Leucine*
Isoleucine*

Phosphoenolpyruvate and erythrose 4-phosphate
Tryptophan*
Phenylalanine*
Tyrosine†

Ribose 5-phosphate
Histidine*

*Essential amino acids.
†Derived from phenylalanine in mammals.

The synthesis of glutamate requires the participation of mitochondrial metabolism

Figure 10.6 shows that glutamate is formed from α-ketoglutarate, which can be provided by a partial sequence of the mitochondrial citrate cycle (Fig 10.11).

Pyruvate and oxaloacetate are transported from the cytosol to the mitochondria by specific translocators. Pyruvate is oxidized by **pyruvate dehydrogenase**, and the acetyl-CoA thus generated condenses with oxaloacetate to citrate. This citrate can be converted in the mitochondria via **aconitase**, oxidized further by **NAD-isocitrate dehydrogenase**, and the resultant α-ketoglutarate can be transported into the cytosol by a specific translocator.

But often a major part of the citrate produced in the mitochondria is exported to the cytosol and converted there to α-ketoglutarate by cytosolic isoenzymes of aconitase and NADP isocitrate dehydrogenase. In this case, only a short partial sequence of the citrate cycle is involved in the synthesis of α-ketoglutarate from pyruvate and oxaloacetate. Citrate is released from the mitochondria by a specific translocator in exchange for oxaloacetate.

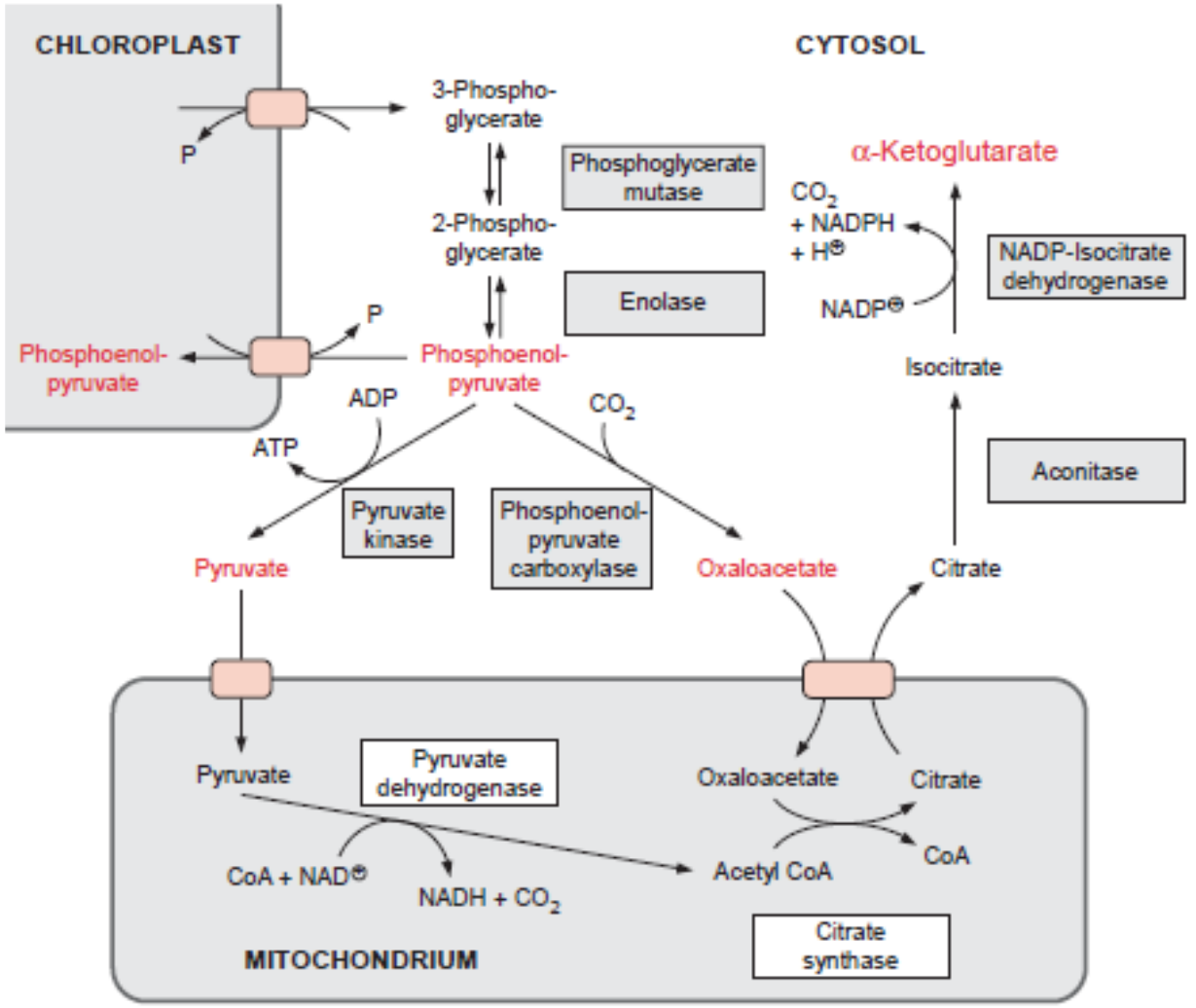


Fig 10.11 Carbon skeletons for the synthesis of amino acids are provided by CO_2 assimilation. Important precursors for amino acid synthesis are colored red.

Biosynthesis of proline and arginine

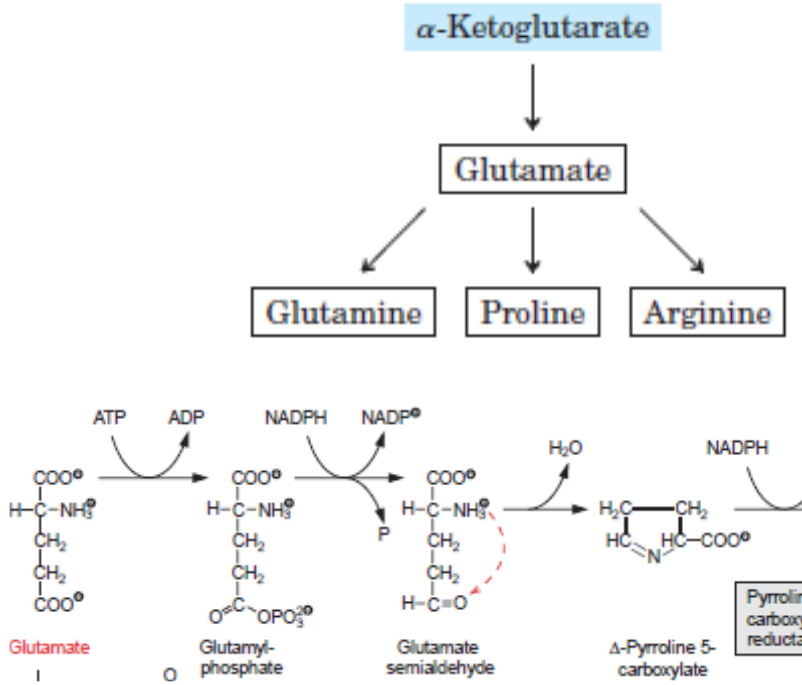
We have already described the biosynthesis of glutamate and glutamine.

Proline is a cyclized derivative of glutamate. In the first step of proline synthesis, ATP reacts with the γ-carboxyl group of glutamate to form an acyl phosphate, which is reduced by NADPH or NADH to glutamate γ-semialdehyde. This intermediate undergoes rapid spontaneous cyclization and is then reduced further to yield proline.

Glutamate is the precursor for the synthesis of **proline**. Its δcarboxylic group is first converted by a **glutamate kinase** to an energy-rich phosphoric acid anhydride and is then reduced by NADPH to an aldehyde.

The accompanying hydrolysis of the energy-rich phosphate drives the reaction, resembling the reduction of 3-phosphoglycerate to glyceraldehyde 3-phosphate in the Calvin cycle. A ring is formed by the condensation of the carbonyl group with the α-amino group. Reduction by NADPH results in the formation of proline.

α-Ketoglutarate Gives Rise to Glutamate, Glutamine, Proline, and Arginine



In the first step of the synthesis of arginine, the α-amino group of glutamate is acetylated by reaction with acetyl-CoA and is thus protected. Subsequently, the d-carboxylic group is phosphorylated and reduced to a semi-aldehyde in basically the same reaction as in proline synthesis. Here the α-amino group is protected and the formation of a ring is not possible.

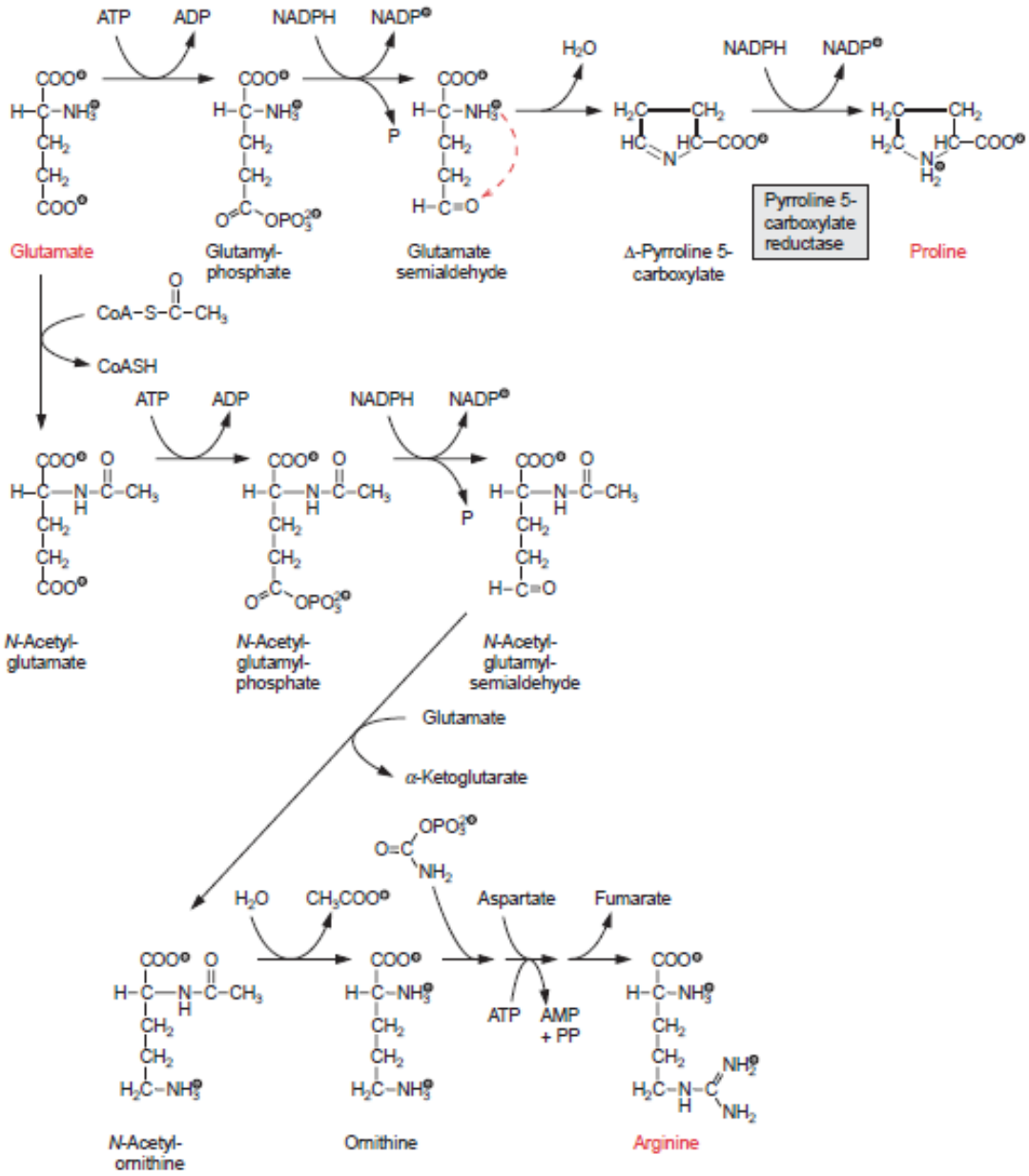
By transamination with glutamate, the aldehyde group is converted to an amino group, and after cleavage of the acetyl residue, ornithine is formed. The conversion of ornithine to arginine (shown only summarily in Fig. 10.12) proceeds in the same way as in the urea cycle of animals, by condensation with carbamoyl phosphate to citrulline. An amino group is transferred from aspartate to citrulline, resulting in the formation of arginine and fumarate.

Biosynthesis of proline and arginine

Glutamate is the precursor for the synthesis of **proline**. Its δcarboxylic group is first converted by a **glutamate kinase** to an energy-rich phosphoric acid anhydride and is then reduced by NADPH to an aldehyde. The accompanying hydrolysis of the energy-rich phosphate drives the reaction, resembling the reduction of 3-phosphoglycerate to glyceraldehyde 3-phosphate in the Calvin cycle. A ring is formed by the condensation of the carbonyl group with the α-amino group. Reduction by NADPH results in the formation of proline.

In the first step of the synthesis of **arginine**, the a-amino group of glutamate is acetylated by reaction with acetyl-CoA and is thus protected. Subsequently, the d-carboxylic group is phosphorylated and reduced to a semi-aldehyde in basically the same reaction as in proline synthesis. Here the a-amino group is protected and the formation of a ring is not possible.

By transamination with glutamate, the aldehyde group is converted to an amino group, and after cleavage of the acetyl residue, ornithine is formed. The conversion of ornithine to arginine (shown only summarily in Fig. 10.12) proceeds in the same way as in the urea cycle of animals, by condensation with carbamoyl phosphate to citrulline. An amino group is transferred from aspartate to citrulline, resulting in the formation of arginine and fumarate.



Arginine is synthesized from glutamate via ornithine and the urea cycle in animals. In principle, ornithine could also be synthesized from glutamate -semialdehyde by transamination, but the spontaneous cyclization of the semialdehyde in the proline pathway precludes a sufficient supply of this intermediate for ornithine synthesis. Bacteria have a de novo biosynthetic pathway for ornithine (and thus arginine) that parallels some steps of the proline pathway but includes two additional steps that avoid the problem of the spontaneous cyclization of glutamate –semialdehyde (Fig. 22–10). In the first step, the -amino group of glutamate is blocked by an acetylation requiring acetyl-CoA; then, after the transamination step, the acetyl group is removed to yield ornithine.

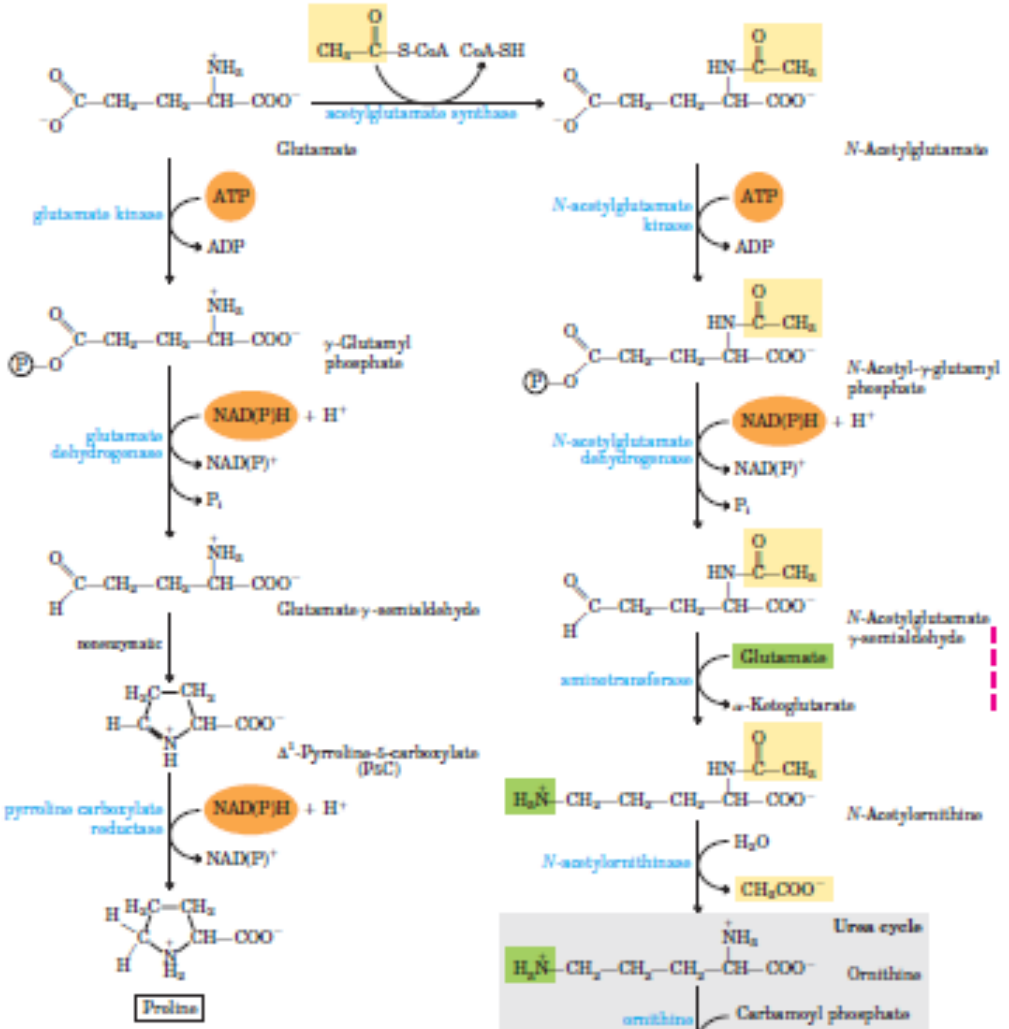


FIGURE 22–10 Biosynthesis of proline and arginine from glutamate in bacteria. All five carbon atoms of proline arise from glutamate. In many organisms, glutamate dehydrogenase is unusual in that it uses either NADH or NADPH as a cofactor. The same may be true of other enzymes in these pathways. The γ-semialdehyde in the proline pathway undergoes a rapid, reversible cyclization to Δ1-pyrroline-5-carboxylate (P5C), with the equilibrium favoring P5C formation. Cyclization is averted in the ornithine/arginine pathway by acetylation of the α-amino group of glutamate in the first step and removal of the acetyl group after the transamination. Although some bacteria lack arginase and thus the complete urea cycle, they can synthesize arginine from ornithine in steps that parallel the mammalian urea cycle, with citrulline and argininosuccinate as intermediates (see Fig. 18–16).

Here, and in subsequent figures in this chapter, the reaction arrows indicate the linear path to the final products, without considering the reversibility of individual steps. For example, the second step of the pathway leading to arginine, catalyzed by N-acetylglutamate

The pathways to proline and arginine are somewhat different **in mammals**. *Proline can be* synthesized by pathway shown in Fig 22–10, but it is *also formed from arginine obtained from dietary or tissue protein*.

Arginase, a urea cycle enzyme, converts arginine to ornithine and urea. The ornithine is converted to glutamate - semialdehyde by the enzyme ornithine-aminotransferase (Fig. 22–11). The semialdehyde cyclizes to 1-pyrroline-5-carboxylate, which is then converted to proline (Fig. 22–10).

The pathway for arginine synthesis shown in Figure 22–10 is absent in mammals.

When arginine from dietary intake or protein turnover is insufficient for protein synthesis, the ornithine - aminotransferase reaction operates in the direction of ornithine formation. Ornithine is then converted to citrulline and arginine in urea cycle.

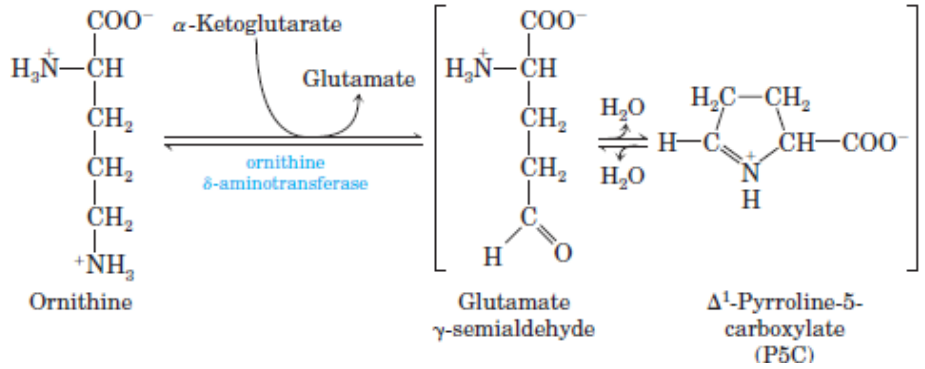
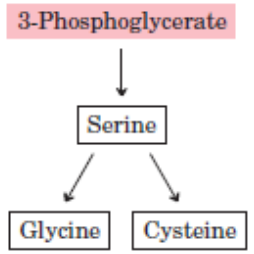


FIGURE 22–11 Ornithine δ–aminotransferase reaction: a step in the mammalian pathway to proline. This enzyme is found in the mitochondrial matrix of most tissues. Although the equilibrium favors P5C formation, the reverse reaction is the only mammalian pathway for synthesis of ornithine (and thus arginine) when arginine levels are insufficient for protein synthesis.

Serine, Glycine, and Cysteine Are Derived from 3-Phosphoglycerate

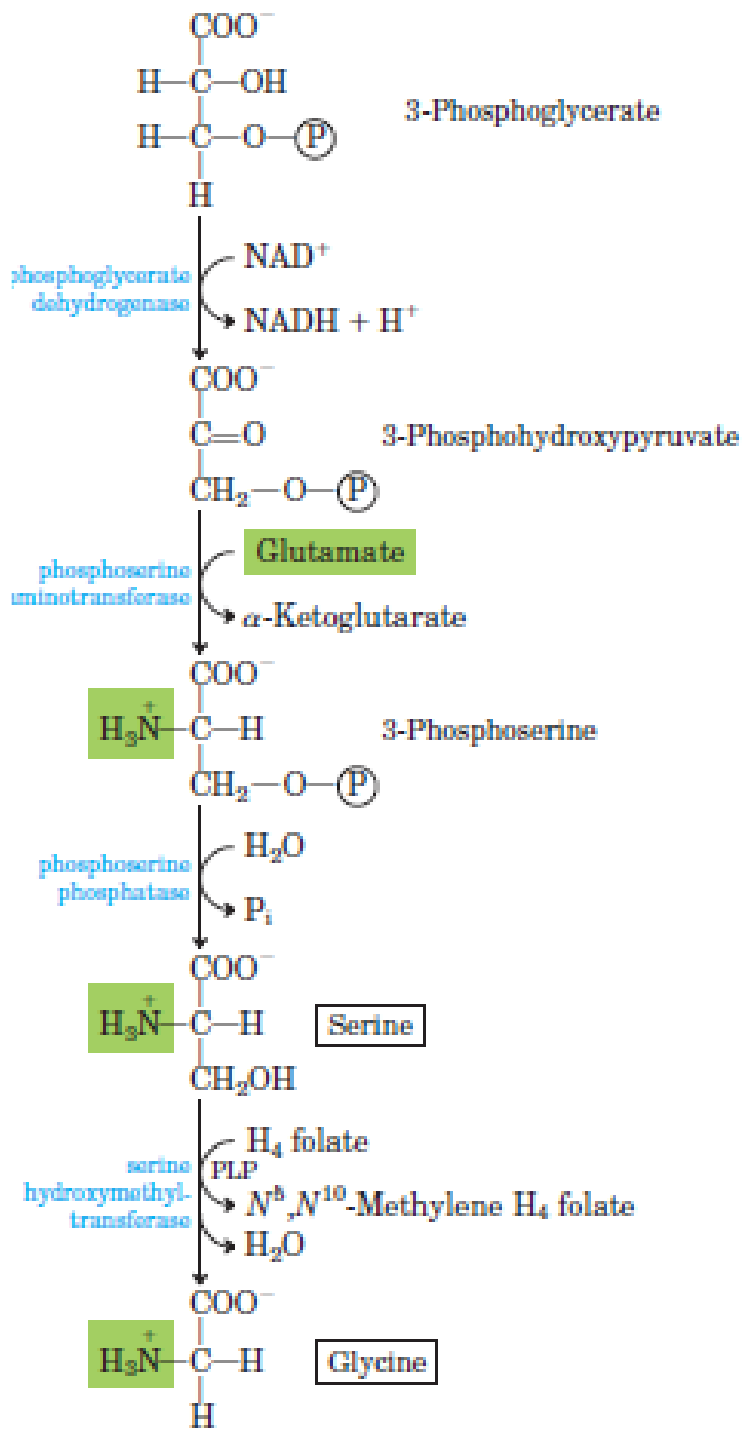
The major pathway for the formation of **serine** is the same in all organisms.



- In the first step, the hydroxyl group of **3-phosphoglycerate** is oxidized by a *dehydrogenase* (using NAD) to yield **3-phosphohydroxypyruvate**.
- Transamination from glutamate yields **3-phosphoserine**, which is hydrolyzed to free **serine** by *phosphoserine phosphatase*.
- Serine (three carbons) is the precursor of **glycine** (two carbons) through removal of a carbon atom by *serine hydroxymethyltransferase*.
- Tetrahydrofolate accepts the carbon (C-3) of serine, which forms a methylene bridge between N-5 and N-10 to yield **N5,N10-methylenetetrahydrofolate**. The overall reaction, which is reversible, also requires pyridoxal phosphate.

- In the **liver of vertebrates**, glycine can be made by another route: the reverse of the reaction, catalyzed by *glycine synthase* (also called glycine cleavage enzyme):

$$CO_2 + NH_4^+ + N^5,N^{10}\text{-methylenetetrahydrofolate} + NADH + H^+ \longrightarrow \text{glycine} + \text{tetrahydrofolate} + NAD^+$$



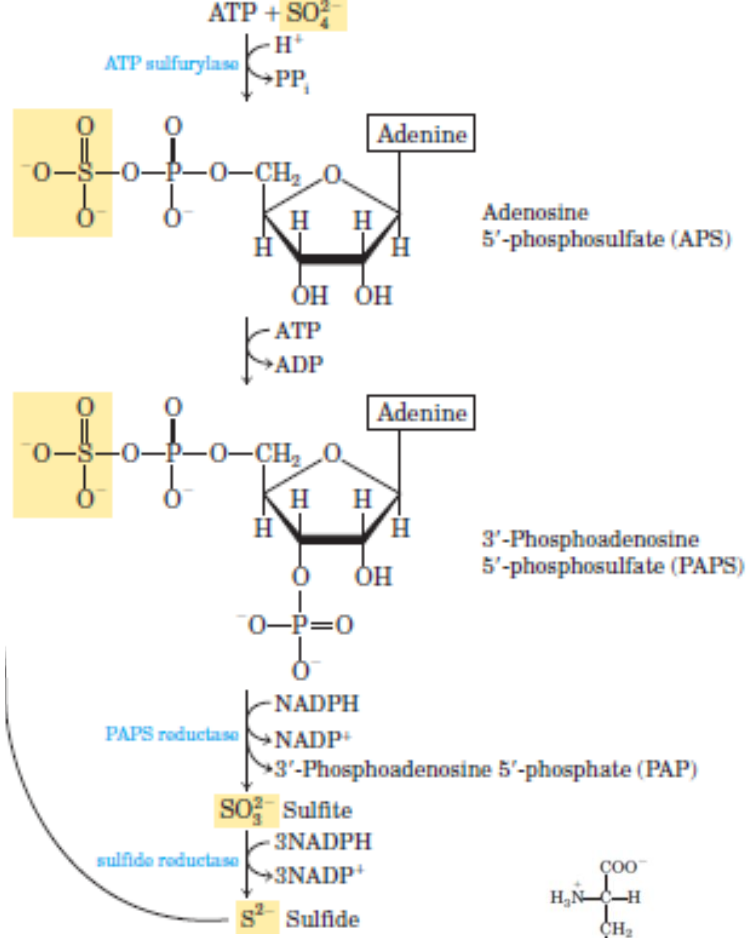
Plants and bacteria produce the reduced sulfur required for the **synthesis of cysteine** (and methionine, described later) from environmental sulfates.

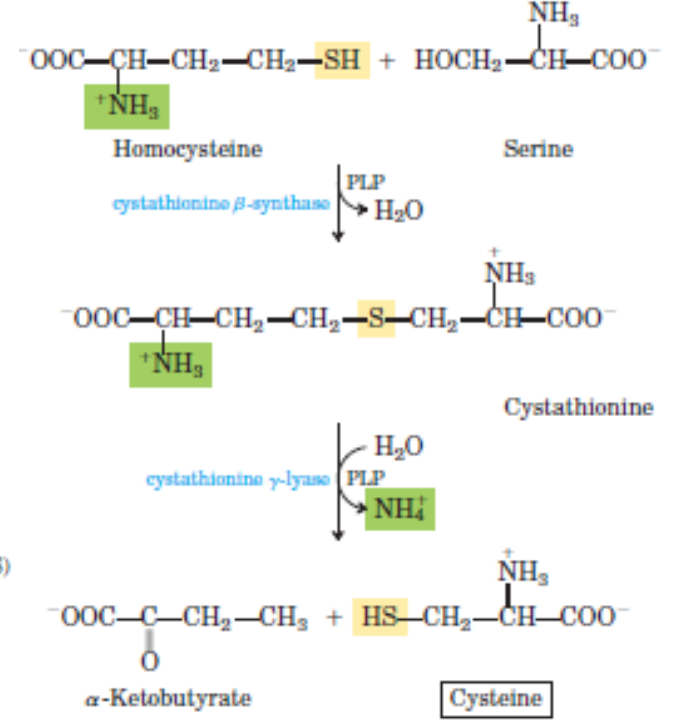
Sulfate is activated in two steps to produce **3-phosphoadenosine 5-phosphosulfate (PAPS)**, which undergoes an eight-electron reduction to **sulfide**. The sulfide is then used in formation of cysteine from serine in a two-step pathway.

Mammals synthesize cysteine from two amino acids: methionine furnishes the sulfur atom and **serine** furnishes the carbon skeleton.

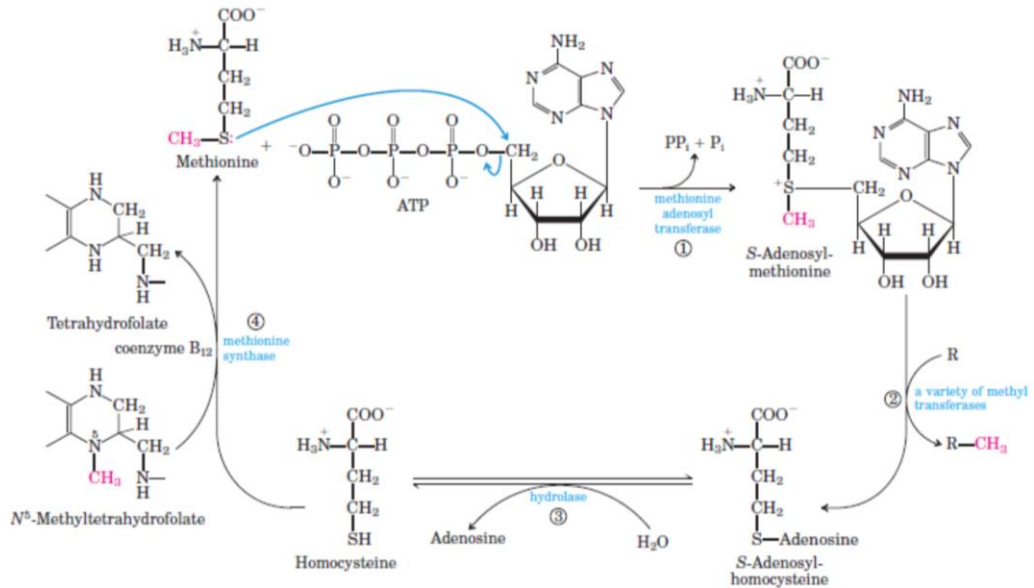
Methionine is first converted to **S-adenosylmethionine** (see Fig. 18–18), which can lose its methyl group to any of a number of acceptors to form S-adenosylhomocysteine (adoHcy). This demethylated product is hydrolyzed to free homocysteine, which undergoes a reaction with serine, catalyzed by **cystathionine β-synthase,** to yield cystathionine (Fig. 22–14).

Finally, **cystathionine γ-lyase,** a PLP requiring enzyme, catalyzes removal of ammonia and cleavage of cystathionine to yield free **cysteine**.





URE 22–14 Biosynthesis of cysteine from homocysteine and ser- in mammals. The homocysteine is formed from methionine, as de- ibed in the text.



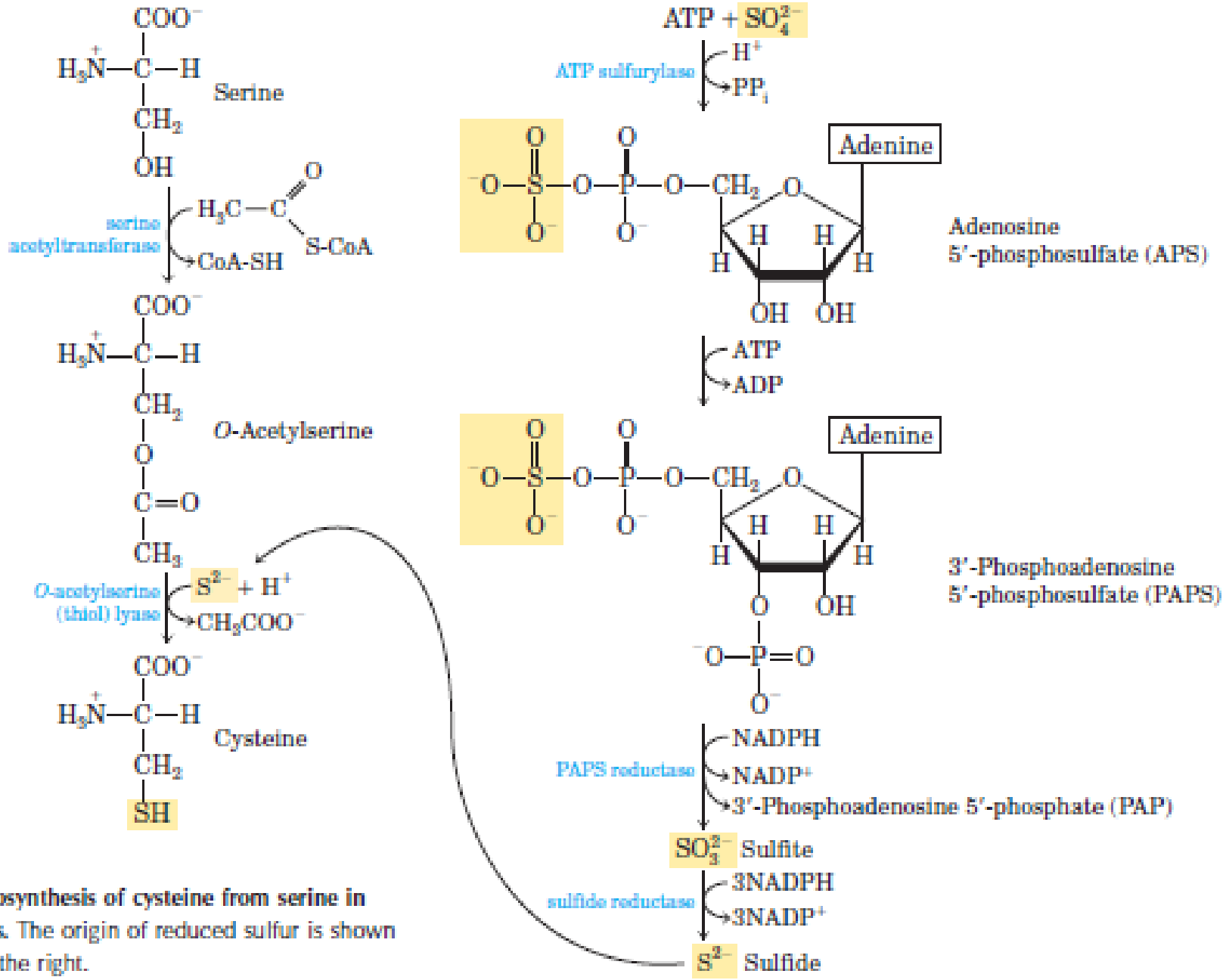


FIGURE 22–13 Biosynthesis of cysteine from serine in bacteria and plants. The origin of reduced sulfur is shown in the pathway on the right.

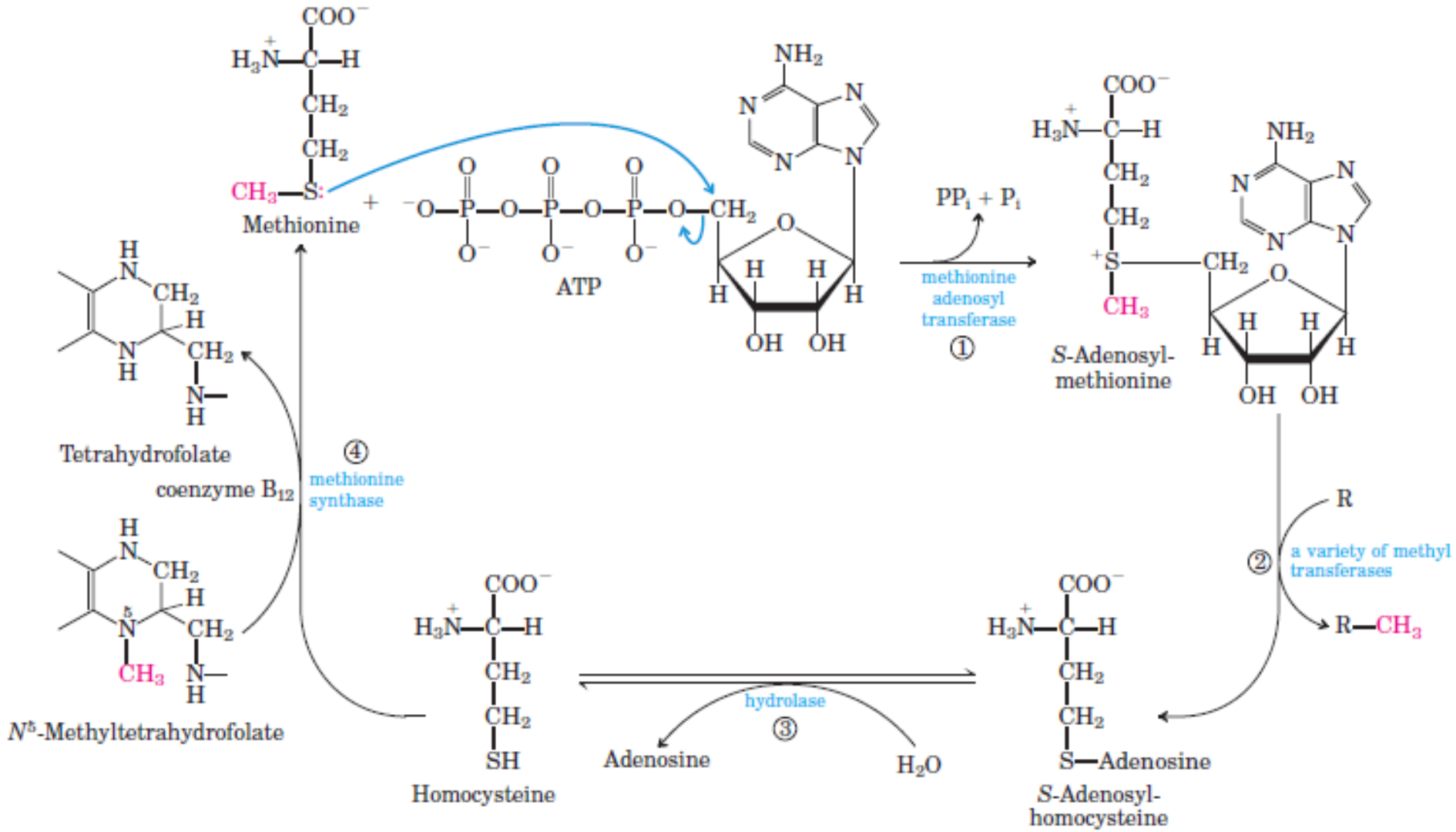


FIGURE 18–18 Synthesis of methionine and *S*-adenosylmethionine in an activated-methyl cycle. The steps are described in the text. In the methionine synthase reaction (step ④), the methyl group is transferred to cobalamin to form methylcobalamin, which in turn is the methyl donor in the formation of methionine. *S*-Adenosylmethionine, which has a positively charged sulfur (and is thus a sulfonium ion), is a powerful methylating agent in a number of biosynthetic reactions. The methyl group acceptor (step ②) is designated R.

Three Nonessential and Six Essential Amino Acids Are Synthesized from Oxaloacetate and Pyruvate

- Alanine and aspartate are synthesized from pyruvate and oxaloacetate, respectively, by transamination from glutamate. Asparagine is synthesized by amidation of aspartate, with glutamine donating the $NH4^+$. These are nonessential amino acids, and their simple biosynthetic pathways occur in all organisms.
- **Methionine, threonine, lysine, isoleucine, valine, and leucine are essential amino acids**. Their biosynthetic pathways are complex and interconnected (Fig. 22–15).
- In some cases, the pathways in bacteria, fungi, and plants differ significantly. The bacterial pathways Figure 22–15.
- Aspartate gives rise to **methionine, threonine,** and **lysine.**
- Branch points occur at **aspartate β-semialdehyde**, an intermediate in all 3 pathways, and at **homoserine**, a precursor of threonine and methionine.
- Threonine, in turn, is one of the precursors of **isoleucine**.

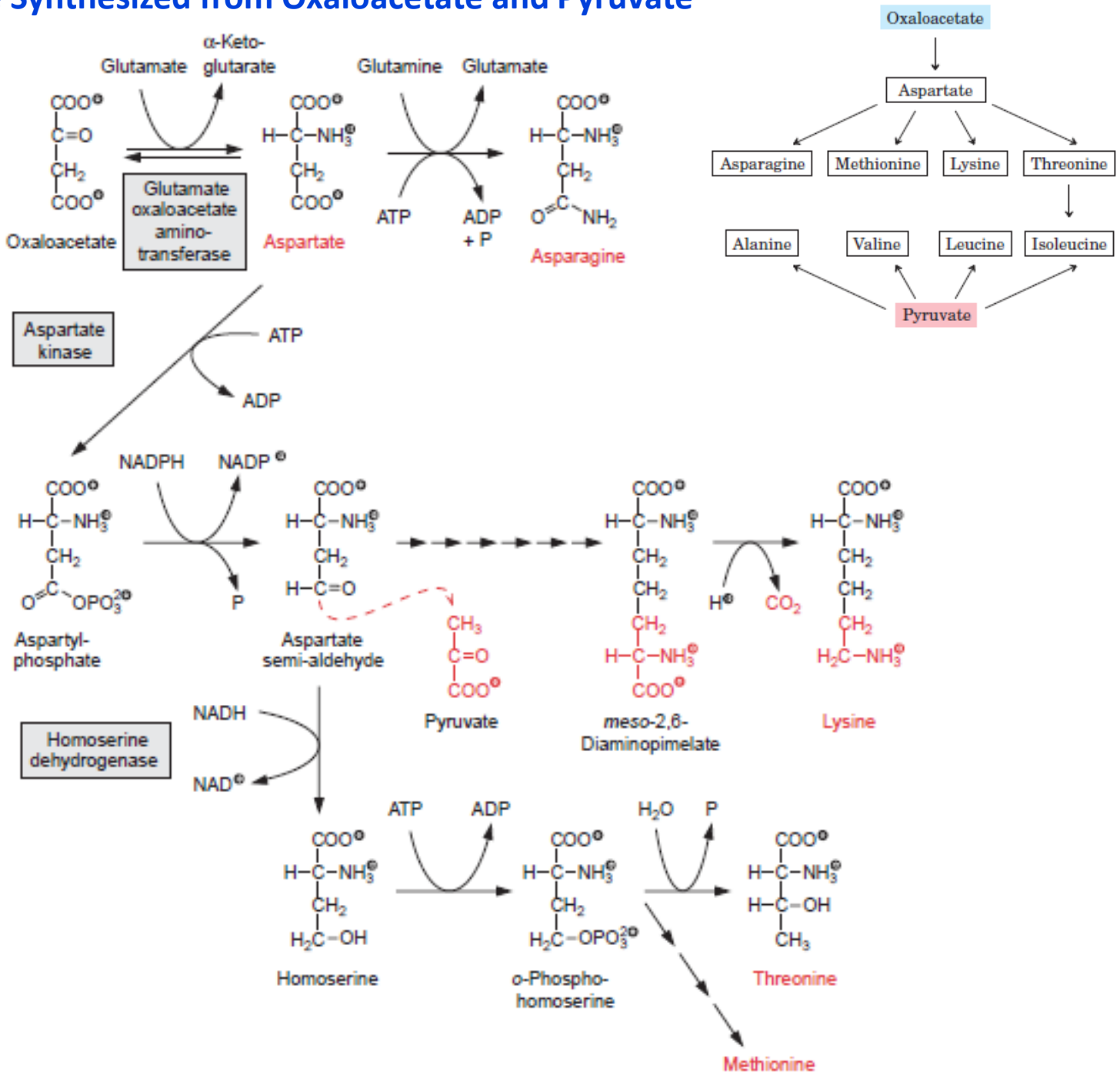
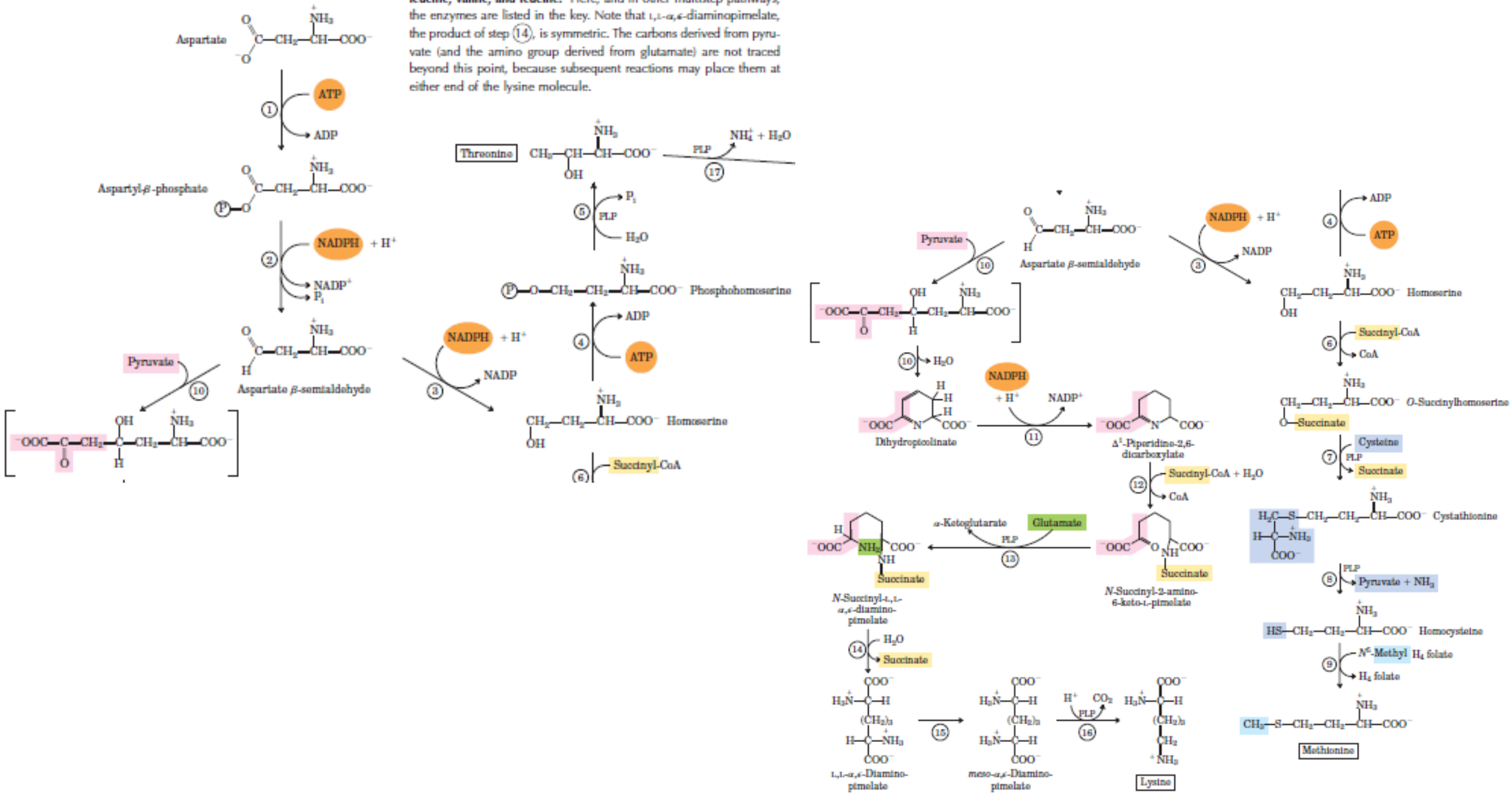
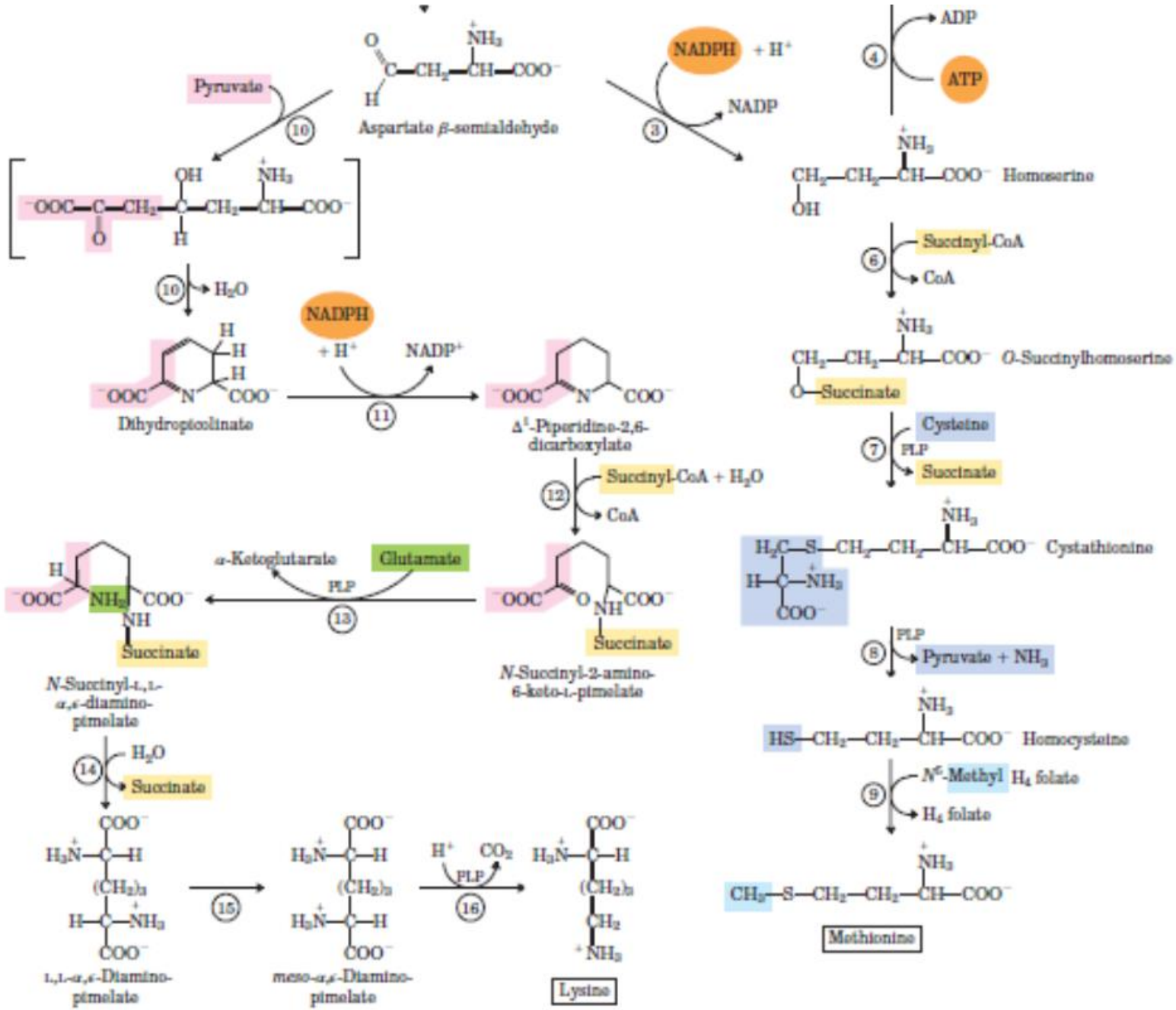


FIGURE 22–15 **Biosynthesis of six essential amino acids from oxaloacetate and pyruvate in bacteria: methionine, threonine, lysine, isoleucine, valine, and leucine.** Here, and in other multistep pathways, the enzymes are listed in the key. Note that L,L-α,ε-diaminopimelate, the product of step (14), is symmetric. The carbons derived from pyruvate (and the amino group derived from glutamate) are not traced beyond this point, because subsequent reactions may place them at either end of the lysine molecule.



Contd…


Pyruvate
10
Aspartate β-semialdehyde
NADPH + H+
NADP
3
4
ADP
ATP
Homoserine
10
H2O
NADPH
+ H+
NADP+
11
Dihydropicolinate
Δ1-Piperidine-2,6-dicarboxylate
6
Succinyl-CoA
CoA
O-Succinylhomoserine
Succinate
7
Cysteine
PLP
Succinate
Cystathionine
12
Succinyl-CoA + H2O
CoA
α-Ketoglutarate
Glutamate
PLP
13
N-Succinyl-2-amino-6-keto-L-pimelate
N-Succinyl-L,L-α,ε-diamino-pimelate
8
PLP
Pyruvate + NH3
Homocysteine
14
H2O
Succinate
9
N5-Methyl H4 folate
H4 folate
L,L-α,ε-Diamino-pimelate
15
meso-α,ε-Diamino-pimelate
H+
CO2
PLP
16
Lysine
Methionine

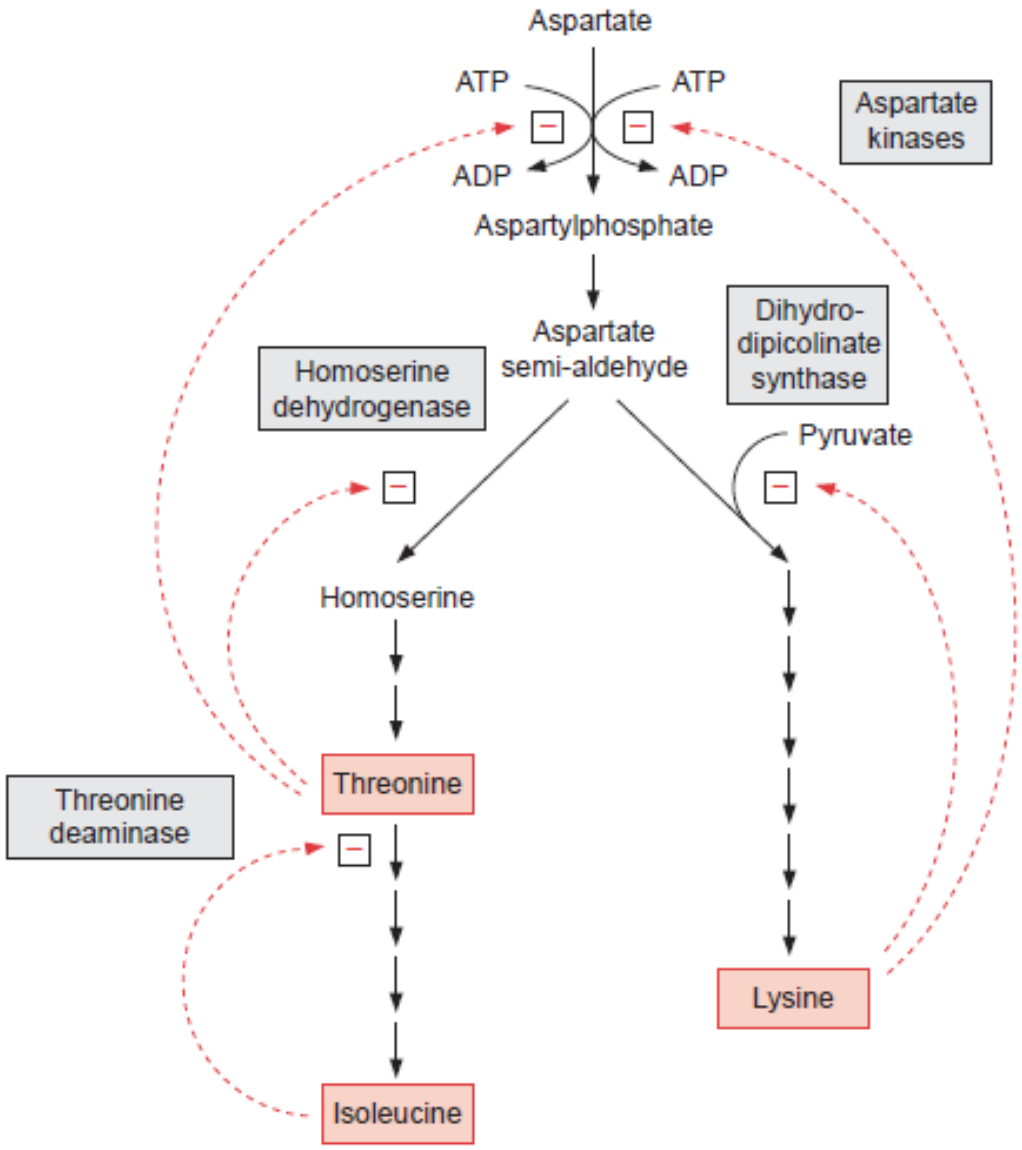


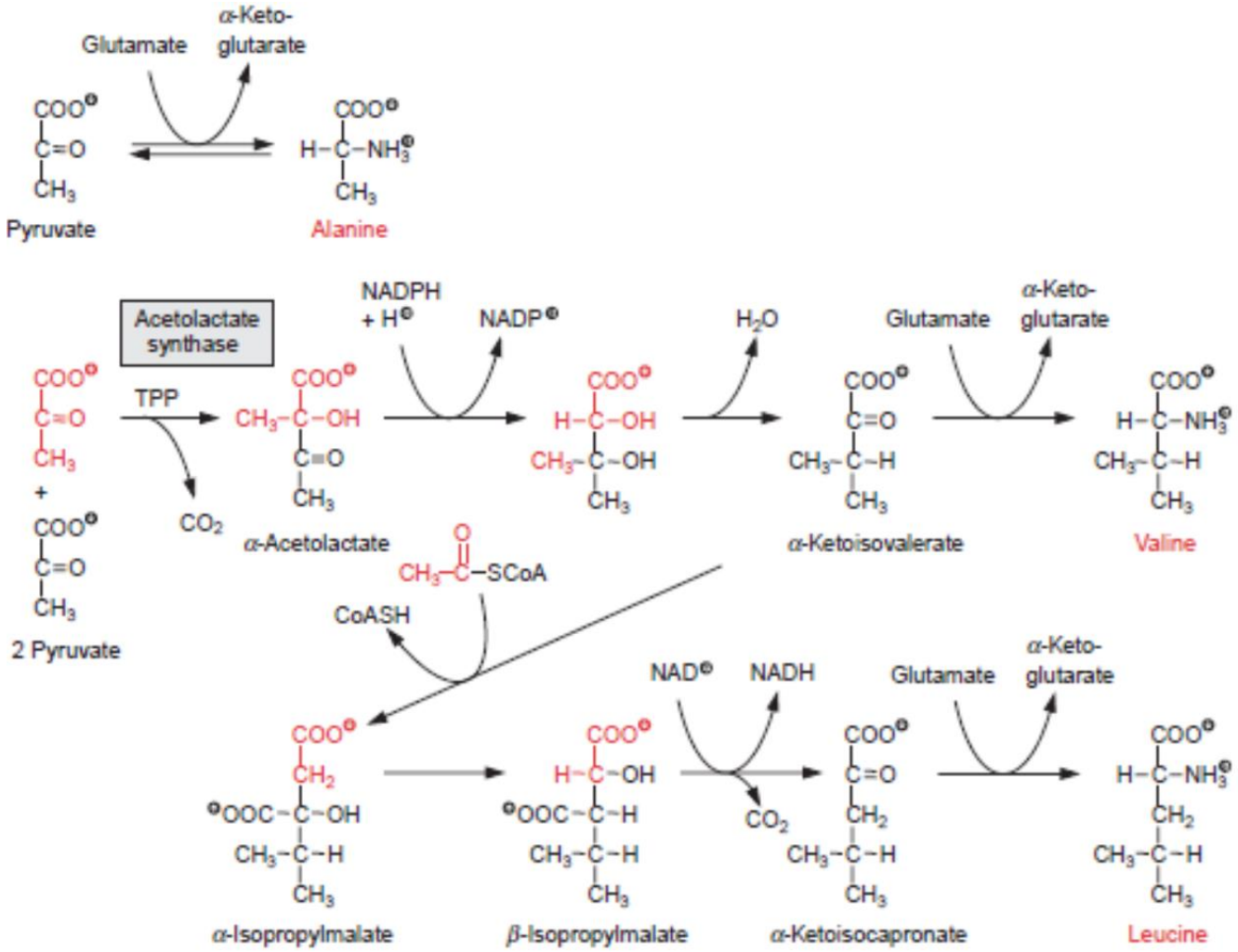
Figure 10.15 Feedback inhibition by end products regulates the entrance enzyme for the synthesis of amino acids from aspartate according to demand. [–] indicates inhibition. Aspartate kinase exists in two isoforms.

Synthesis of amino acids from aspartate is subject to strong feedback control by its end products (Fig. 10.15). Aspartate kinase, the entrance valve for the synthetic pathways, is present in two isoforms. One is inhibited by threonine and the other by lysine. In addition, the reactions of aspartate semi-aldehyde at the branch points of both synthetic pathways are inhibited by the corresponding end products.

Acetolactate synthase participates in the synthesis of hydrophobic amino acids

Pyruvate can be converted by transamination to **alanine** (Fig. 10.16A).

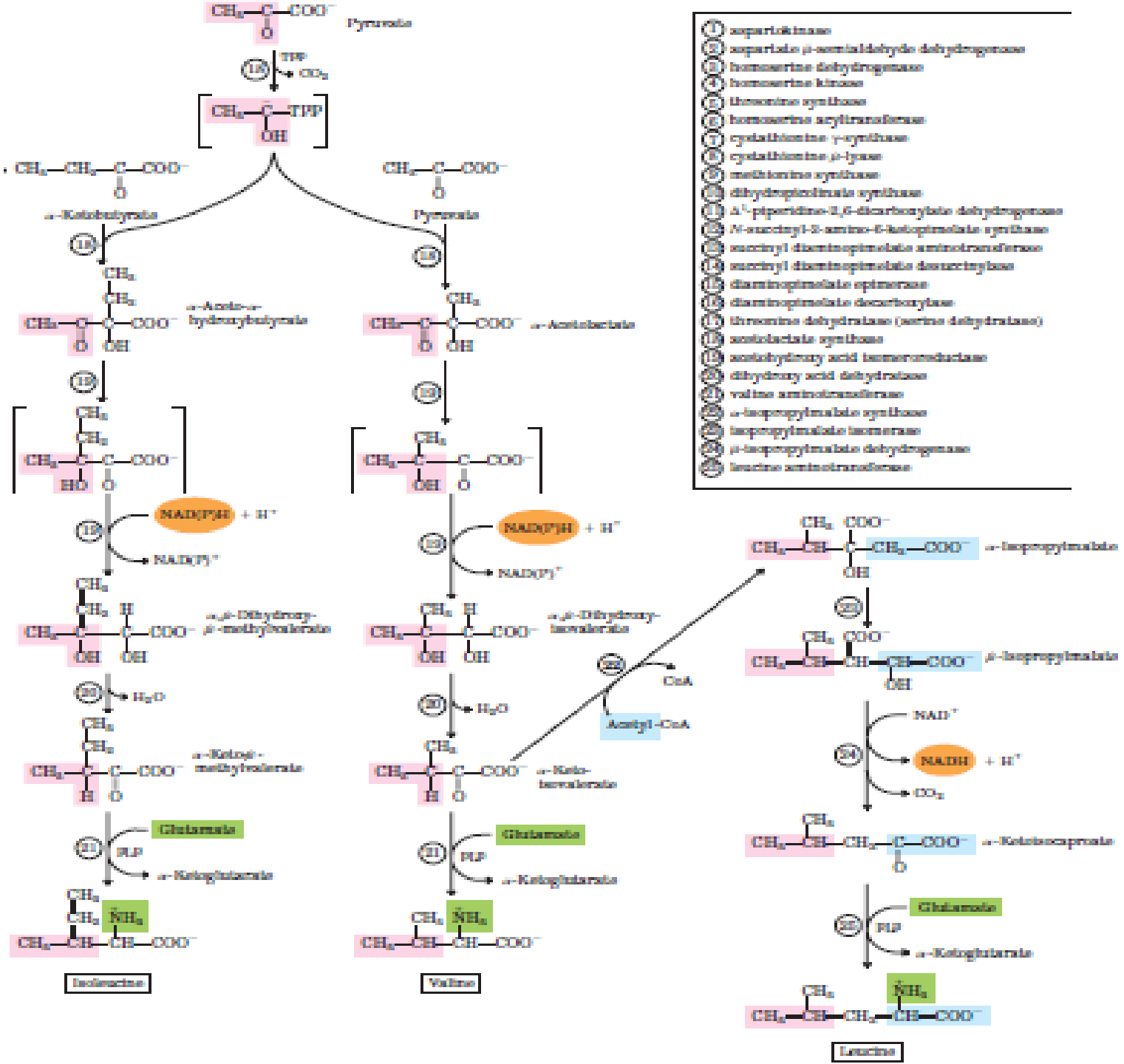
Synthesis of **valine** and **leucine** begins with the formation of acetolactate from two molecules of pyruvate. ***Acetolactate synthase***, catalyzing this reaction, contains **thiamine pyrophosphate (TPP)** as its prosthetic group. The reaction of TPP with pyruvate yields hydroxyethyl-TPP and CO_2. The hydroxyethyl residue is transferred to a second molecule of pyruvate and thus **acetolactate** is formed. Its reduction and rearrangement and the release of water yields α-ketoisovalerate and a subsequent transamination by glutamate produces **valine**.

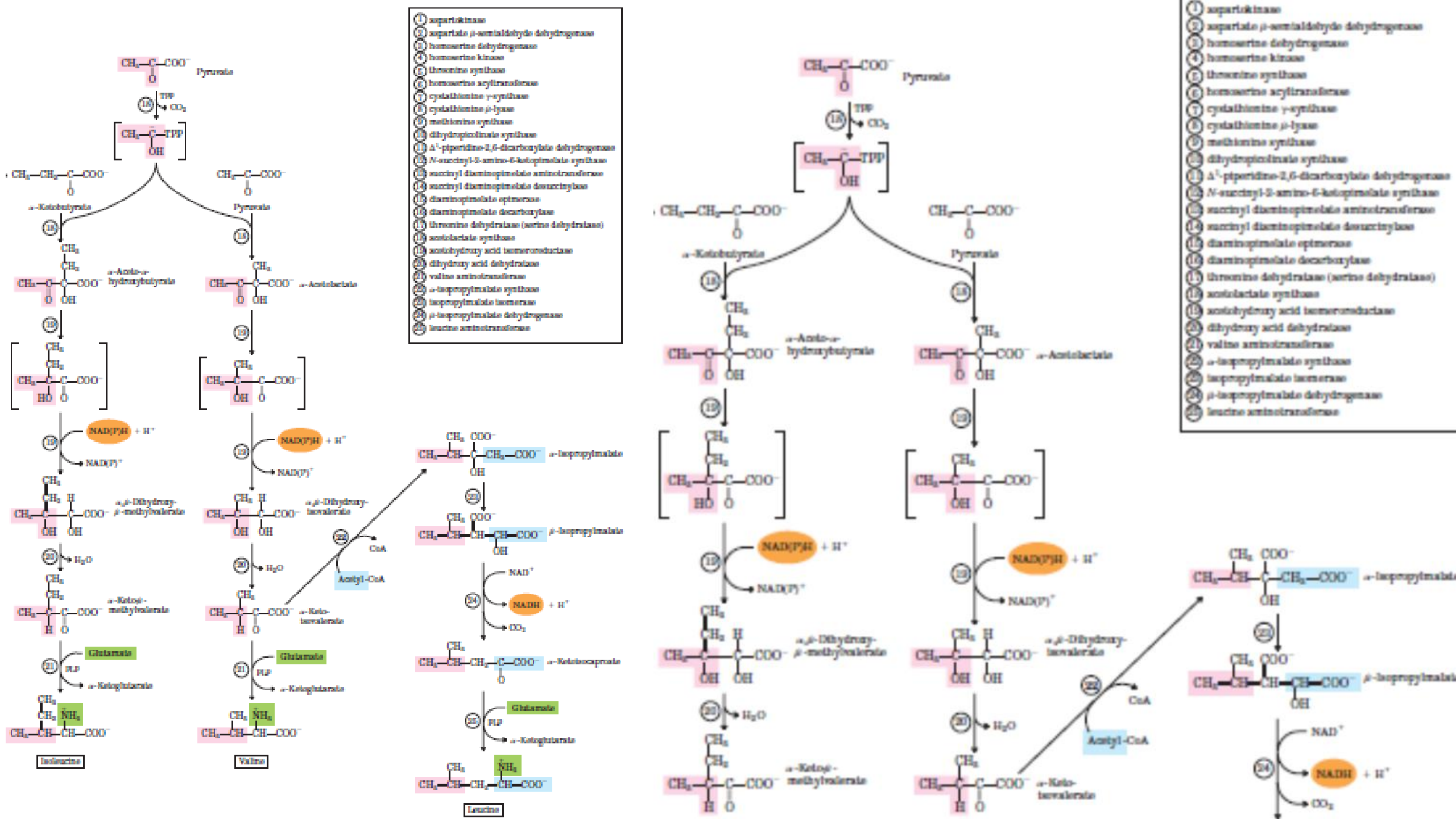


The formation of leucine from α-ketoisovalerate proceeds in basically the same reaction sequences as for the formation of glutamate from oxaloacetate. First, acetyl-CoA condenses with α –ketoisovalerate, the product α -isopropylmalate isomerizes, and the β-isopropylmalate thus formed is oxidized by NAD+ with the release of CO_2 to α ketoisocapronate. Finally, in analogy to the synthesis of glutamate, α ketoisocapronate is transformed by transamination to leucine.

For the synthesis of isoleucine from threonine, the latter is first converted by a deaminase to α-ketobutyrate. Acetolactate synthase condenses α-ketobutyrate with pyruvate in a reaction analogous to the synthesis of acetolactate from two molecules of pyruvate. The further reactions in the synthesis of isoleucine correspond to the reaction sequence in the synthesis of valine.

The **valine** and **isoleucine** pathways share four enzymes (Fig. 22–15, steps 18 to 21). Pyruvate gives rise to valine and isoleucine in pathways that begin with condensation of two carbons of pyruvate (in the form of hydroxyethyl thiamine pyrophosphate) with another molecule of pyruvate (valine path) or with -ketobutyrate (isoleucine path). The -ketobutyrate is derived from threonine in a reaction that requires pyridoxal phosphate. An intermediate in the valine pathway, -ketoisovalerate, is the starting point for a four-step branch pathway leading to **leucine** (steps 22 to 25).

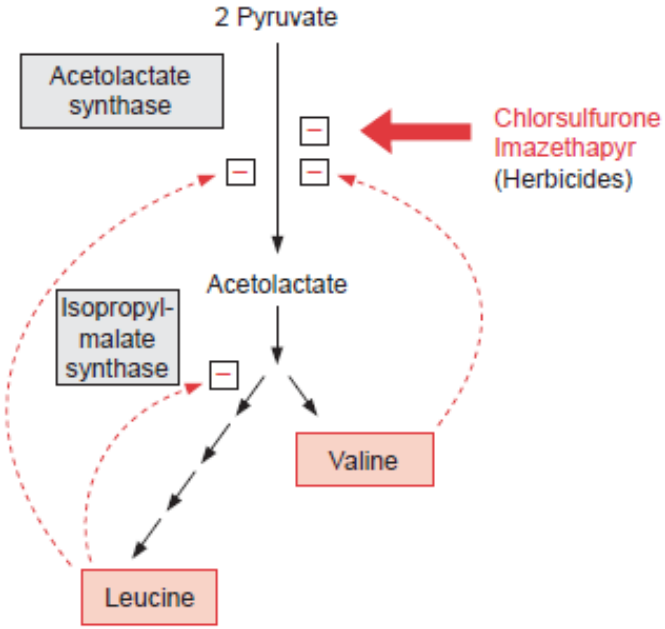


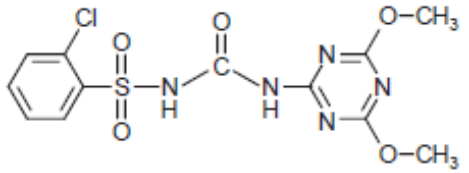

1 aspartokinase
2 aspartate β-semialdehyde dehydrogenase
3 homoserine dehydrogenase
4 homoserine kinase
5 threonine synthase
6 homoserine acyltransferase
7 cystathionine γ-synthase
8 cystathionine β-lyase
9 methionine synthase
10 dihydropicolinate synthase
11 Δ1-piperidine-2,6-dicarboxylate dehydrogenase
12 N-succinyl-2-amino-6-ketopimelate synthase
13 succinyl diaminopimelate aminotransferase
14 succinyl diaminopimelate desuccinylase
15 diaminopimelate epimerase
16 diaminopimelate decarboxylase
17 threonine dehydratase (serine dehydratase)
18 acetolactate synthase
19 acetohydroxy acid isomeroreductase
20 dihydroxy acid dehydratase
21 valine aminotransferase
22 α-isopropylmalate synthase
23 isopropylmalate isomerase
24 β-isopropylmalate dehydrogenase
25 leucine aminotransferase
Pyruvate
TPP
CO2
α-Ketobutyrate
α-Aceto-α-hydroxybutyrate
α-Acetolactate
α,β-Dihydroxy-β-methylvalerate
α,β-Dihydroxyisovalerate
α-Keto-β-methylvalerate
α-Ketoisovalerate
NAD(P)H + H+
NAD(P)+
H2O
Glutamate
PLP
α-Ketoglutarate
Isoleucine
Valine
CoA
Acetyl-CoA
α-Isopropylmalate
β-Isopropylmalate
α-Ketoisocaproate
NAD+
NADH + H+
CO2
Leucine

The synthesis of leucine, valine, and isoleucine is also subject to feedback control by the end products. Isopropylmalate synthase is inhibited by leucine (Fig. 10.17) and threonine deaminase is inhibited by isoleucine (Fig. 10.15).

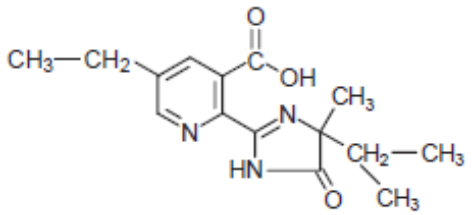
The first enzyme, acetolactate synthase (ALS), is inhibited by valine and leucine. **Sulfonyl ureas, (e.g., chlorsulfurone) and imidazolinones, (e.g., imazethapyr)** (Fig. 10.18) are very strong inhibitors of ALS, where they bind to the pyruvate binding site. A concentration as low as 10^{-9} mol/L of chlorsulfurone is sufficient to inhibit ALS by 50%. Since the pathway for the formation of valine, leucine, and isoleucine is present only in plants and microorganisms, the inhibitors aforementioned are suitable for destroying plants specifically and are therefore used as **herbicides**. Chlorsulfurone (trade name Glean, DuPont) is used as a selective herbicide in the cultivation of cereals, and imazethapyr (Pursuit, American Cyanamide Co.) is used for protecting soybeans. From the application of these herbicides, mutants of maize, soybean, rapeseed, and wheat have naturally evolved, which are resistant to sulfonyl ureas or imidazolinones, or even to both herbicides. In each case, a mutation was found in the gene for acetolactate synthase, making the enzyme insensitive to the herbicides without affecting its enzyme activity. By crossing these mutants with other lines, herbicide-resistant varieties have been bred and are, in part, already commercially cultivated.

Figure 10.17 Synthesis of valine and leucine is adjusted to demand by the inhibitory effect of both amino acids on acetolactate synthase and the inhibition of isopropyl malate synthase by leucine. The herbicides chlorsulfurone and imazethapyr inhibit acetolactate synthase. [–] indicates inhibition.

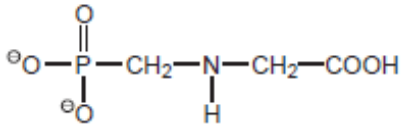




Chlorsulfurone



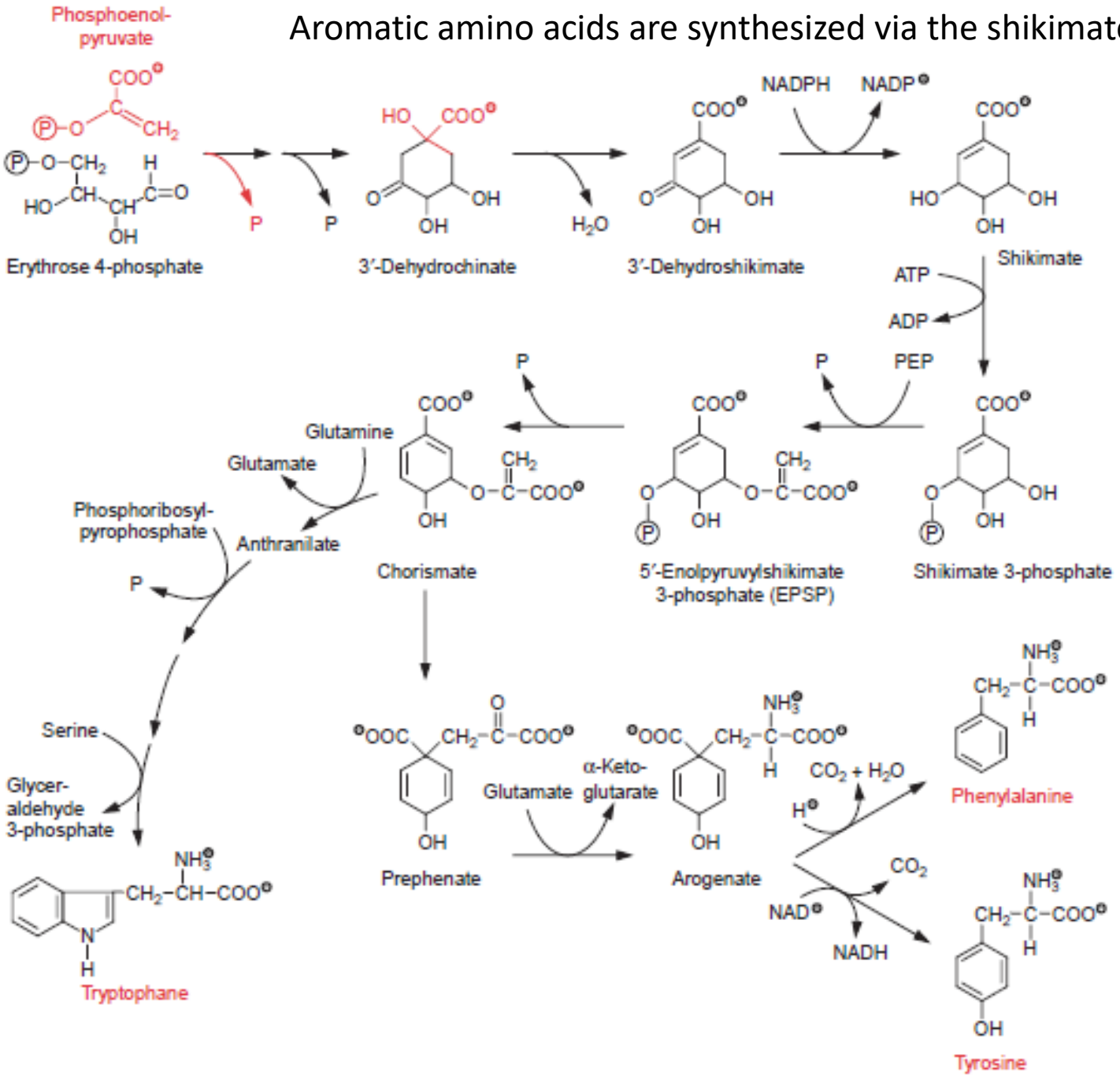
Imazethapyr

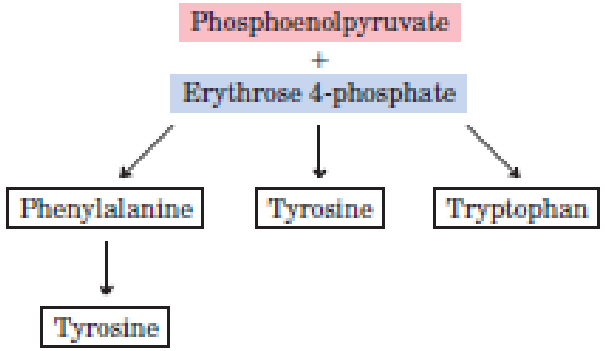


Glyphosate

Chorismate Is a Key Intermediate in the Synthesis of Tryptophan, Phenylalanine, and Tyrosine

Aromatic amino acids are synthesized via the shikimate pathway





Precursors for aromatic amino acids formation are erythrose 4-phosphate and PEP, that condense to form **cyclic dehydrochinate** accompanied by phosphate groups liberation. Following water removal and reduction of carbonyl group, **shikimate** is formed. After protection of 3¢-hydroxyl group by phosphorylation, the 5¢-hydroxyl group of shikimate reacts with PEP to give **enolether 5¢-enolpyruvyl shikimate-3-phosphate (EPSP)** and chorismate is formed by phosphate removal. Chorismate represents a branch point for 2 biosynthetic pathways.

The first four steps produce ***shikimate***, a seven-carbon molecule derived from erythrose 4-phosphate and phosphoenolpyruvate. Shikimate is converted to ***chorismate*** in 3 steps that include addition of 3 more carbons from another molecule of PEP. Chorismate is the first branch point of pathway, with one branch leading to tryptophan, other to phenylalanine and tyrosine.

Aromatic rings are not readily available in the environment, even though the benzene ring is very stable. The branched pathway to tryptophan, phenylalanine, and tyrosine, occurring in bacteria, fungi, and plants, is the main biological route of aromatic ring formation. It proceeds through ring closure of an aliphatic precursor followed by stepwise addition of double bonds.

In the tryptophan branch, chorismite is converted to **anthranilate** in a reaction in which glutamine donates the nitrogen that will become part of the indole ring. Anthranilate then condenses with PRPP. The indole ring of tryptophan is derived from the ring carbons and amino group of anthranilate plus two carbons derived from PRPP. The final reaction in the sequence is catalyzed by ***tryptophan synthase***. This enzyme has an 22 subunit structure and can be dissociated into two subunits and a 2 subunit that catalyze different parts of the overall reaction:

$$\text{Indole-3-glycerol phosphate} \xrightarrow[\alpha \text{ subunit}]{} \text{indole} + \text{glyceraldehyde 3-phosphate}$$

$$\text{Indole} + \text{serine} \xrightarrow[\beta_2 \text{ subunit}]{} \text{tryptophan} + H_2O$$

1. anthranilate synthase
2. anthranilate phosphoribosyltransferase
3. N-(5'-phosphoribosyl)-anthranilate isomerase
4. indole-3-glycerol phosphate synthase
5. tryptophan synthase

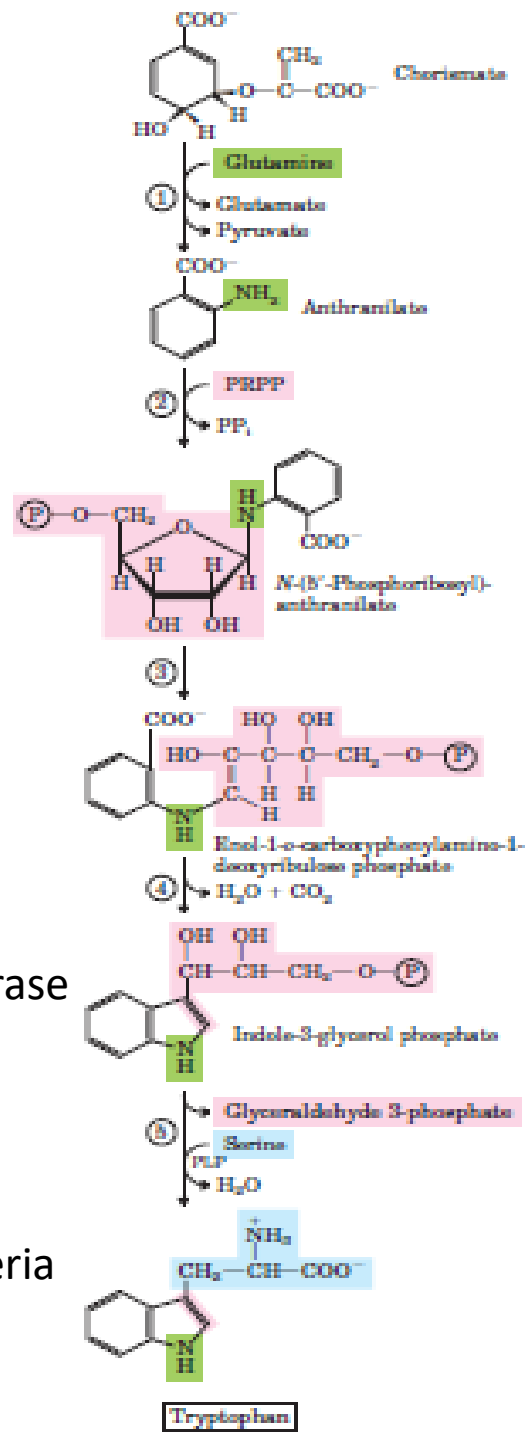
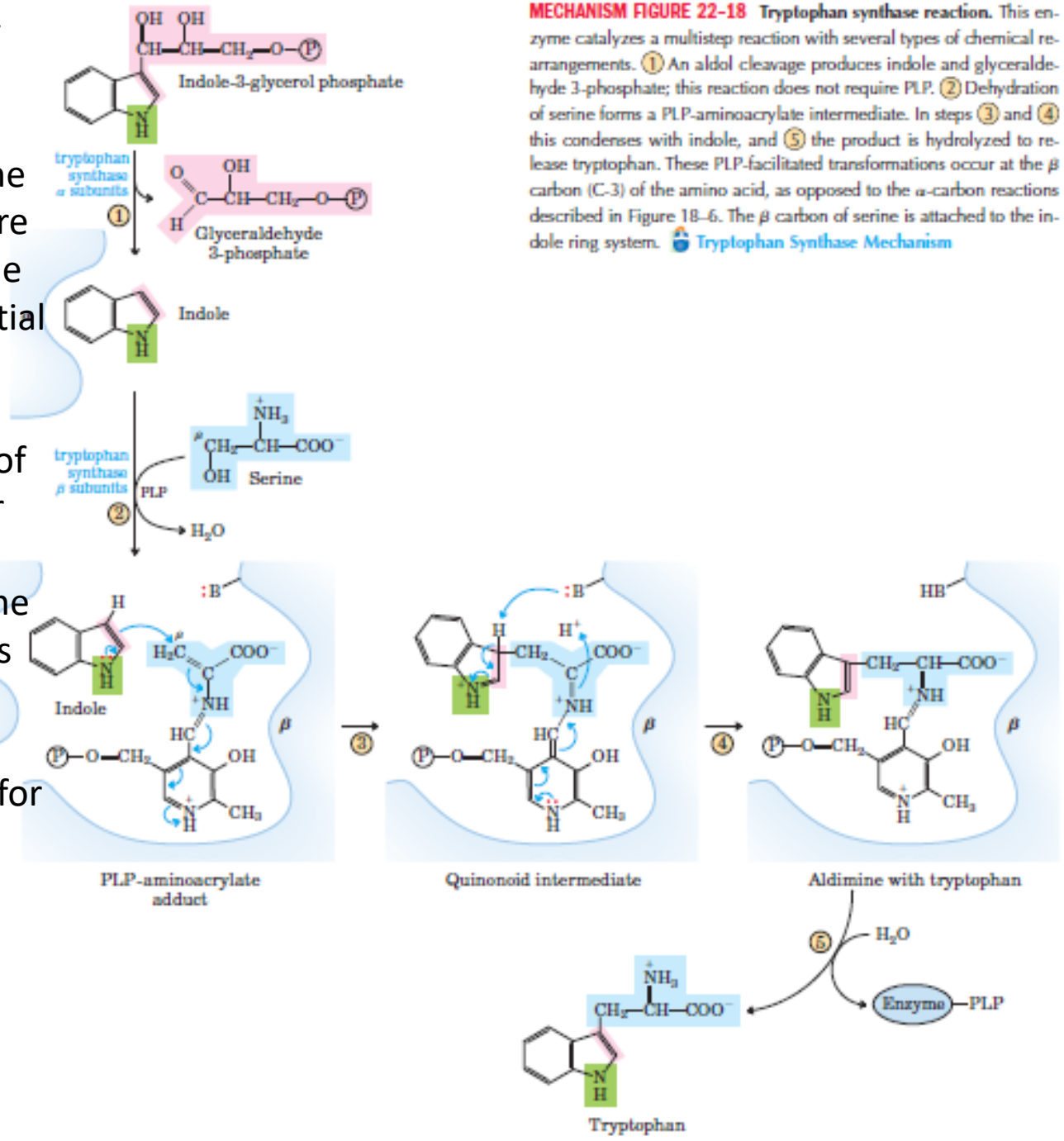


FIGURE Biosynthesis of tryptophan from chorismate in bacteria and plants. In E. coli, enzymes catalyzing steps 1 and 2 are subunits of a single complex.

The second part of the reaction requires pyridoxal phosphate (Fig. 22–18). Indole formed in the first part is not released by the enzyme, but instead moves through a channel from the -subunit active site to the –subunit active site, where it condenses with a Schiff base intermediate derived from serine and PLP. Intermediate channeling of this type may be a feature of the entire pathway from chorismate to tryptophan. Enzyme active sites catalyzing different steps (sometimes not sequential steps) of the pathway to tryptophan are found on single polypeptides in some species of fungi and bacteria, but are separate proteins in others. In addition, the activity of some of these enzymes requires a noncovalent association with other enzymes of the pathway. These observations suggest that all the pathway enzymes are components of a large, multienzyme complex in both prokaryotes and eukaryotes. Such complexes are generally not preserved intact when the enzymes are isolated using traditional biochemical methods, but evidence for the existence of multienzyme complexes is accumulating for this and a number of other metabolic pathways.

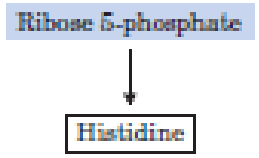


MECHANISM FIGURE 22–18 **Tryptophan synthase reaction.** This enzyme catalyzes a multistep reaction with several types of chemical rearrangements. ① An aldol cleavage produces indole and glyceraldehyde 3-phosphate; this reaction does not require PLP. ② Dehydration of serine forms a PLP-aminoacrylate intermediate. In steps ③ and ④ this condenses with indole, and ⑤ the product is hydrolyzed to release tryptophan. These PLP-facilitated transformations occur at the β carbon (C-3) of the amino acid, as opposed to the α-carbon reactions described in Figure 18–6. The β carbon of serine is attached to the indole ring system. Tryptophan Synthase Mechanism

In plants and bacteria, **phenylalanine** and **tyrosine** are synthesized from chorismate in pathways much less complex than the tryptophan pathway. The common intermediate is prephenate (Fig. 22–19). The final step in both cases is transamination with glutamate.

Animals can produce tyrosine directly from phenylalanine through hydroxylation at C-4 of the phenyl group by **phenylalanine hydroxylase**; this enzyme also participates in the degradation of phenylalanine. Tyrosine is considered a conditionally essential amino acid, or as nonessential insofar as it can be synthesized from the essential amino acid phenylalanine.

Histidine Biosynthesis Uses Precursors of Purine Biosynthesis



The pathway to histidine in all plants and bacteria differs in several respects from other amino acid biosynthetic pathways. Histidine is derived from three precursors (Fig. 22–20): PRPP contributes five carbons, the purine ring of ATP contributes a nitrogen and a carbon, and glutamine supplies the second ring nitrogen. The key steps are condensation of ATP and PRPP, in which N-1 of the purine ring is linked to the activated C-1 of the ribose of PRPP (step 1 in Fig. 22–20); purine ring opening that ultimately leaves N-1 and C-2 of adenine linked to the ribose (step 3); and formation of the imidazole ring, a reaction in which glutamine donates a nitrogen (step 5). The use of ATP as a metabolite rather than a high-energy cofactor is unusual— but not wasteful, because it dovetails with the purine biosynthetic pathway. The remnant of ATP that is released after the transfer of N-1 and C-2 is 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), an intermediate of purine biosynthesis that is rapidly recycled to ATP.

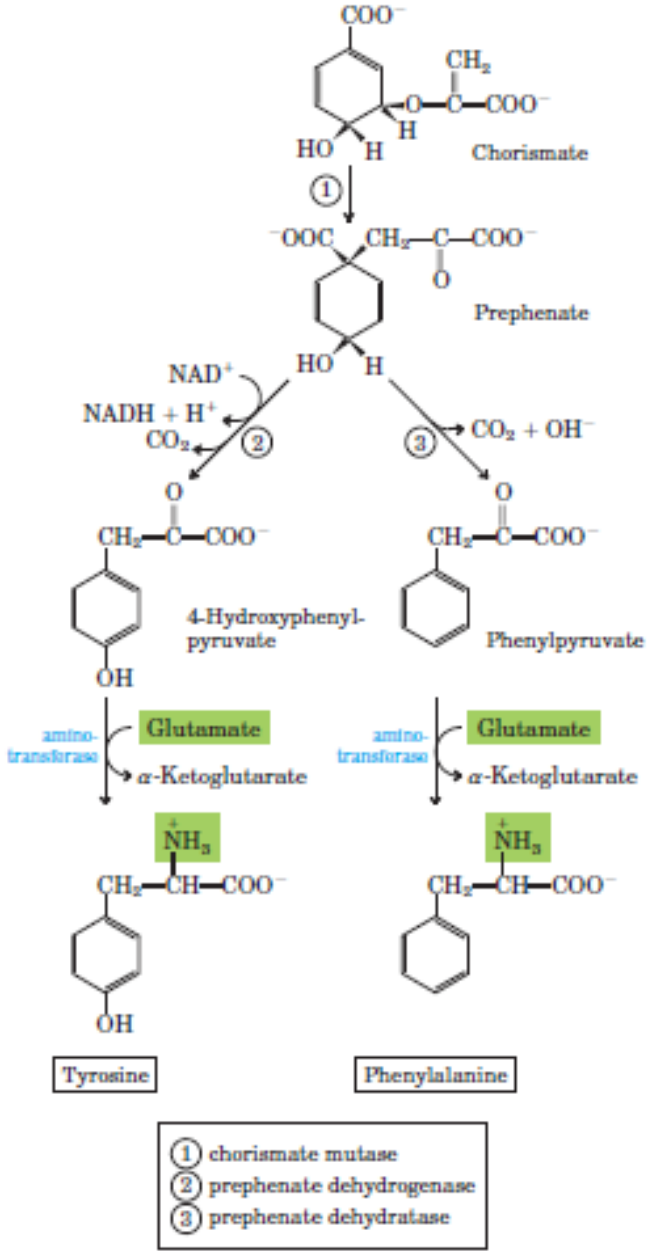


FIGURE 22–19 Biosynthesis of phenylalanine and tyrosine from chorismite in bacteria and plants. Conversion of chorismate to prephenate is a rare biological example of a Claisen rearrangement.

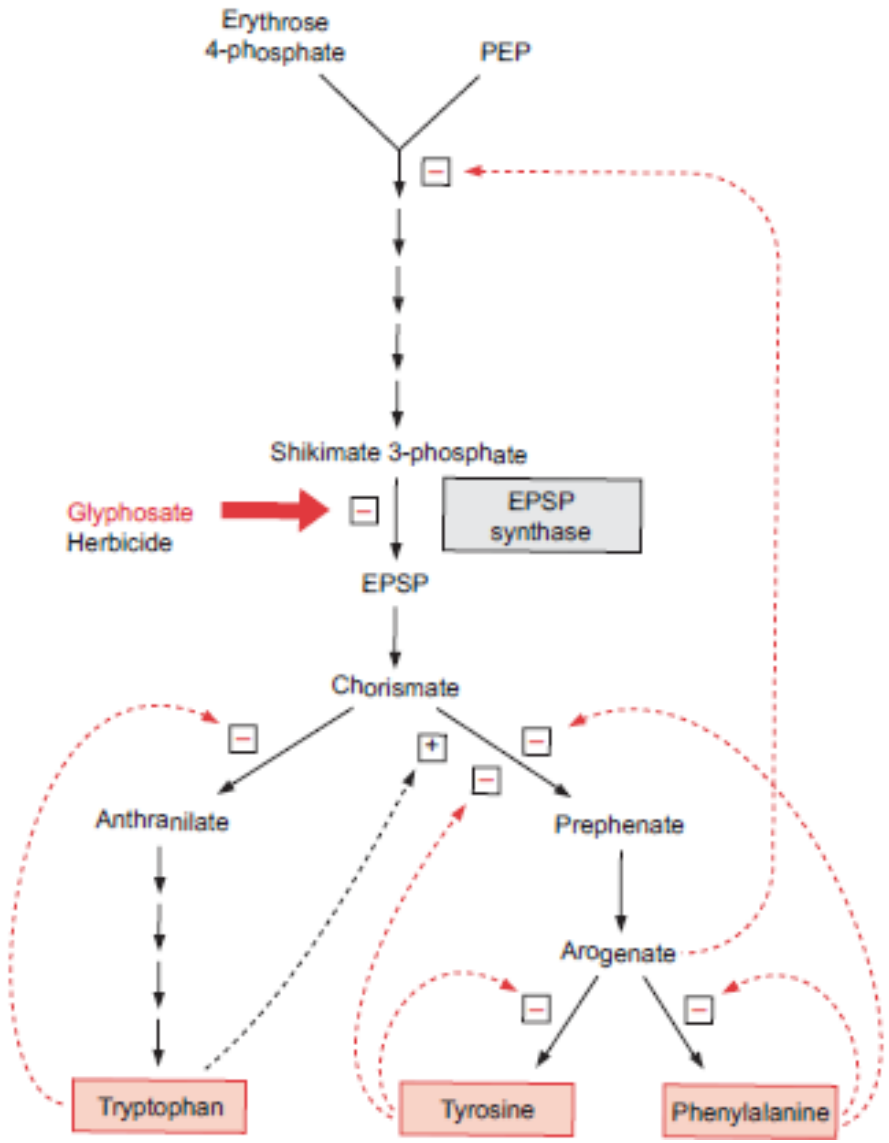


Figure 10.20 Several steps in the synthesis of aromatic amino acids are regulated by product feedback inhibition, thus adjusting the rate of synthesis to demand. Tryptophan stimulates the synthesis of tyrosine and phenylalanine [+]. The herbicide glyphosate (Fig. 10.19) inhibits EPSP synthase [–].

SUMMARY 22.2 Biosynthesis of Amino Acids

- Plants and bacteria synthesize all 20 common amino acids. Mammals can synthesize about half; the others are required in the diet (essential amino acids).
- Among the nonessential amino acids, glutamate is formed by reductive amination of -ketoglutarate and serves as the precursor of glutamine, proline, and arginine. Alanine and aspartate (and thus asparagine) are formed from pyruvate and oxaloacetate, respectively, by transamination. The carbon chain of serine is derived from 3-phosphoglycerate. Serine is a precursor of glycine; the -carbon atom of serine is transferred to tetrahydrofolate. In microorganisms, cysteine is produced from serine and from sulfide produced by the reduction of environmental sulfate. Mammals produce cysteine from methionine and serine by a series of reactions requiring S-adenosylmethionine and cystathionine.
- Among the essential amino acids, the aromatic amino acids (phenylalanine, tyrosine, and tryptophan) form by a pathway in which chorismate occupies a key branch point. Phosphoribosyl pyrophosphate is a precursor of tryptophan and histidine. The pathway to histidine is interconnected with the purine synthetic pathway. Tyrosine can also be formed by hydroxylation of phenylalanine (and thus is considered conditionally essential). The pathways for the other essential amino acids are complex.
- The amino acid biosynthetic pathways are subject to allosteric end-product inhibition; the regulatory enzyme is usually the first in the sequence. Regulation of the various synthetic pathways is coordinated.

(Note: All the original contributors of the concept and findings published elsewhere are gratefully acknowledged while preparing the E-content for the purpose of student reading material in convenient form for biochemistry and allied discipline).

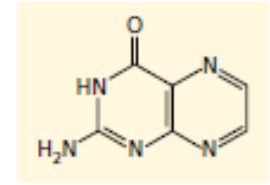
References

- Taiz, L. and Zeiger, E. (2006) Plant physiology. 4th Edition, Sinauer Associates, Inc., Sunderland.

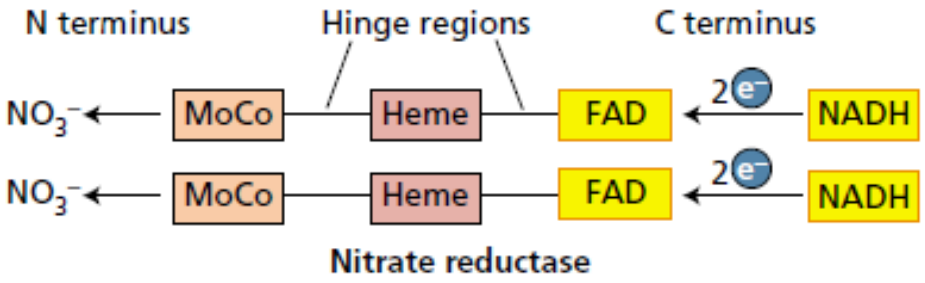
- Hans-Walter Heldt and Fiona Heldt (2005) Plant Biochemistry. 3rd Edition, Elsevier Academic Press, USA.

NITRATE ASSIMILATION

- Plants assimilate most of nitrate absorbed by their roots into organic nitrogen compounds. The **first step** of this process is the reduction of nitrate to nitrite in cytosol. The enzyme **nitrate reductase** catalyzes the reaction
  NO_3^- + NAD(P)H + H+ + 2 e– → NO_2^- + NAD(P)+ + H2O
  where NAD(P)H indicates NADH or NADPH. The most common form of nitrate reductase uses only **NADH** as an electron donor; another form of enzyme found predominantly in nongreen tissues such as roots can use either NADH or NADPH.
- Nitrate reductases of higher plants are composed of 2 identical subunits, each containing 3 prosthetic groups: **FAD** (flavin adenine dinucleotide), **heme**, and a ***molybdenum complexed to an organic molecule*** called a ***pterin***.
- Nitrate reductase is the main molybdenum-containing protein in vegetative tissues, and one symptom of Mo deficiency is accumulation of nitrate that results from diminished nitrate reductase activity.
- Comparison of amino acid sequences for nitrate reductase from several species with those of other well characterized proteins that bind FAD, heme, or molybdenum has led to 3-domain model for nitrate reductase shown below. FAD-binding domain accepts 2 electrons from NADH or NADPH The electrons then pass through the heme domain to the molybdenum complex, where they are transferred to nitrate.



A pterin (fully oxidized)

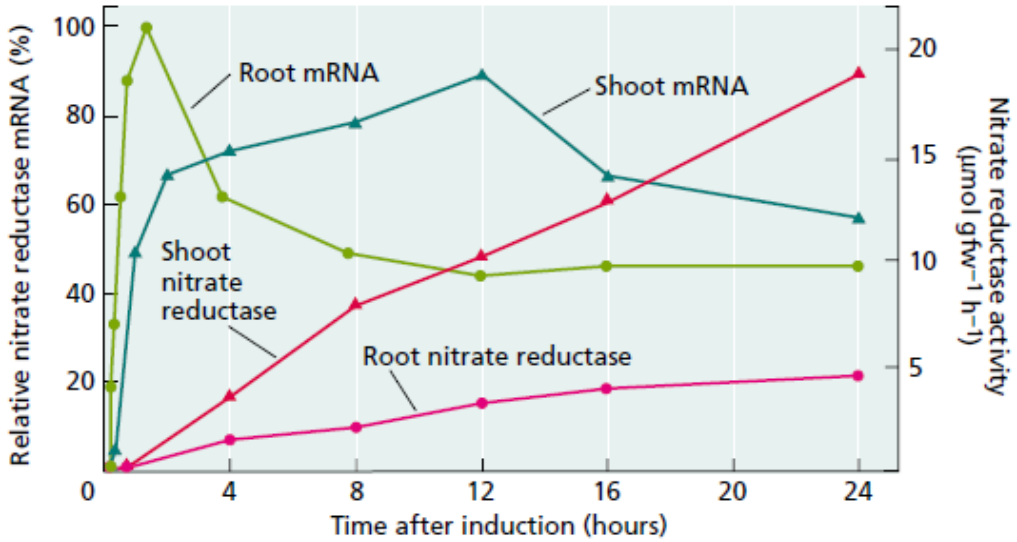


A model of the **nitrate reductase dimer**, illustrating 3 binding domains whose polypeptide sequences are similar in eukaryotes: molybdenum complex (MoCo), heme, and FAD. The NADH binds at FAD binding region of each subunit and initiates a 2-electron transfer from the carboxyl (C) terminus, through each of the electron transfer components, to amino (N) terminus. Nitrate is reduced at molybdenum complex near amino terminus.

Nitrate, Light, and Carbohydrates Regulate Nitrate Reductase

- Nitrate, light, and carbohydrates influence nitrate reductase at the transcription and translation levels. In barley seedlings, nitrate reductase mRNA was detected approximately 40 minutes after addition of nitrate, and maximum levels were attained within 3 hours. In contrast to the rapid mRNA accumulation, there was a gradual linear increase in nitrate reductase activity, reflecting the slower synthesis of the protein.
- In addition, the protein is subject to posttranslational modulation (involving a reversible phosphorylation). Light, carbohydrate levels, and other environmental factors stimulate a protein phosphatase that dephosphorylates several serine residues on the nitrate reductase protein and thereby activates the enzyme.
- Operating in the reverse direction, darkness and Mg2+ stimulate a protein kinase that phosphorylates the same serine residues, which then interact with a 14-3-3 inhibitor protein, and thereby inactivate nitrate reductase.
- ***Regulation of nitrate reductase activity through phosphorylation and dephosphorylation provides more rapid control than can be achieved through synthesis or degradation of the enzyme (minutes versus hours).***

Stimulation of nitrate reductase activity follows the induction of nitrate reductase mRNA in shoots and roots of barley; gfw, grams fresh weight.



Nitrite Reductase Converts Nitrite to Ammonium

Nitrite (NO_2^-) is a highly reactive, potentially toxic ion. Plant cells immediately transport the nitrite generated by nitrate reduction (see Equation 12.1) from the cytosol into chloroplasts in leaves and plastids in roots. In these organelles, the enzyme nitrite reductase reduces nitrite to ammonium according to the following overall reaction:

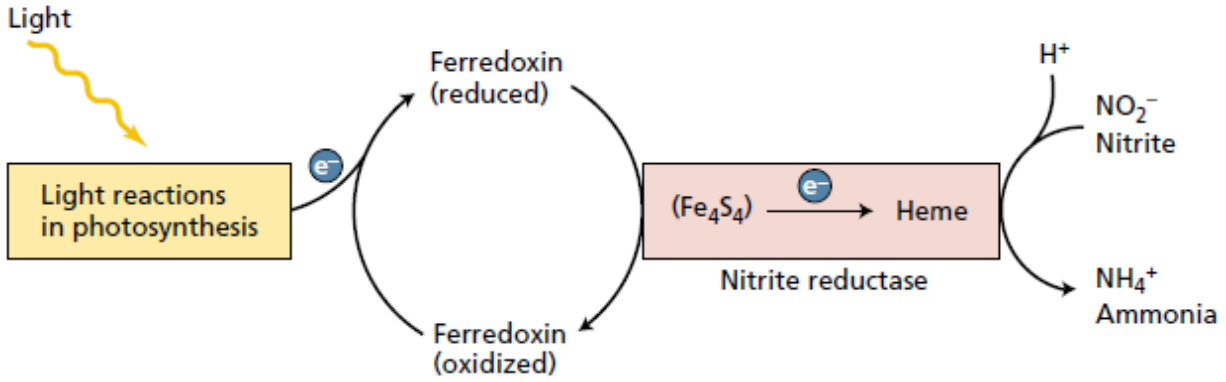
NO2– + 6 Fdred + 8 H+ + 6 e– → NH4 + + 6 Fdox + 2 H2O (12.2)

where Fd is ferredoxin, and the subscripts *red* and *ox* stand for *reduced* and *oxidized*, respectively. Reduced ferredoxin derives from photosynthetic electron transport in the chloroplasts and from NADPH generated by the oxidative pentose phosphate pathway in nongreen tissues.

Chloroplasts and root plastids contain different forms of the enzyme, but both forms consist of a single polypeptide containing 2 prosthetic groups: an iron–sulfur cluster (Fe4S4) and a specialized heme.

These groups acting together bind nitrite and reduce it directly to ammonium, without accumulation of nitrogen compounds of intermediate redox states. The electron flow through ferredoxin (Fe4S4) and heme can be represented as in Figure below.

Nitrite reductase is encoded in the nucleus and synthesized in the cytoplasm with an N-terminal transit peptide that targets it to the plastids. Whereas NO3– and light induce the transcription of nitrite reductase mRNA, the end products of the process—**asparagine and glutamine**—repress this induction.



Model for coupling of photosynthetic electron flow, via ferredoxin, to the reduction of nitrite by nitrite reductase. The enzyme contains two prosthetic groups, Fe_4S_4 and heme, which participate in the reduction of nitrite to ammonium.

Plants Can Assimilate Nitrate in Both Roots and Shoots

In many plants, when the roots receive small amounts of nitrate, nitrate is reduced primarily in the roots. As the supply of nitrate increases, a greater proportion of the absorbed nitrate is translocated to the shoot and assimilated there.

Even under similar conditions of nitrate supply, the balance between root and shoot nitrate metabolism—as indicated by the proportion of nitrate reductase activity in each of the two organs or by the relative concentrations of nitrate and reduced nitrogen in the xylem sap—varies from species to species.

AMMONIUM ASSIMILATION

Plant cells avoid ammonium toxicity by rapidly converting the ammonium generated from nitrate assimilation or photorespiration into amino acids.

The primary pathway for this conversion involves sequential actions of **glutamine synthetase and glutamate synthase.**

Next, we will discuss the enzymatic processes that mediate the assimilation of ammonium into essential amino acids, and the role of amides in the regulation of nitrogen and carbon metabolism.

Conversion of Ammonium to Amino Acids Requires Two Enzymes

- **Glutamine synthetase** (**GS**) combines ammonium with glutamate to form glutamine

Glutamate + NH4 + + ATP ----→ glutamine + ADP + Pi (12.3)

This reaction requires the hydrolysis of one ATP and involves a divalent cation such as Mg2+, Mn2+, or Co2+ as a cofactor. Plants contain **2 classes of GS**, one in **cytosol** and other in **root plastids or shoot chloroplasts**.

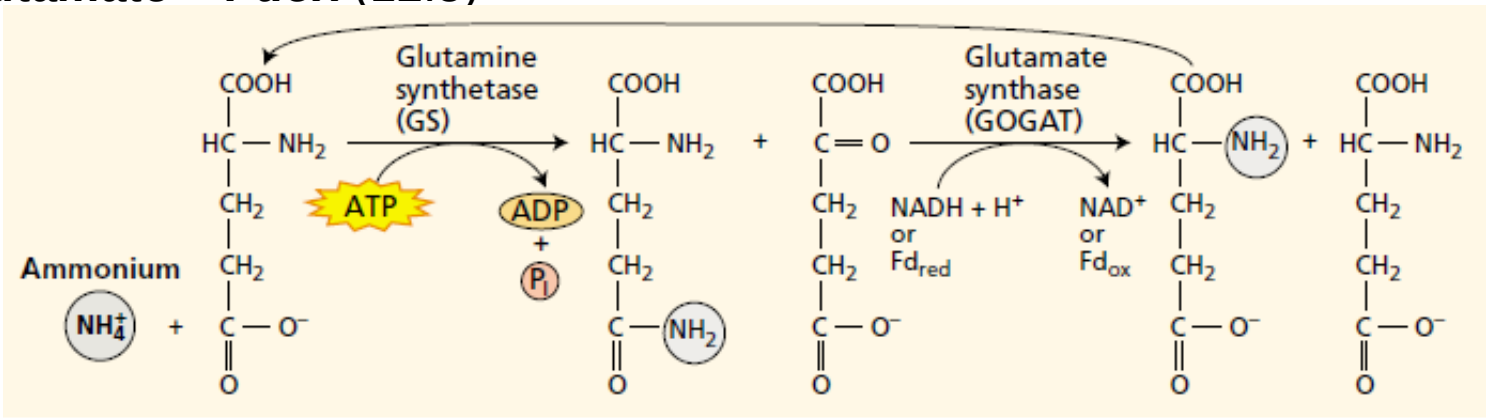
The cytosolic forms are expressed in germinating seeds / vascular bundles of roots and shoots and produce glutamine for intracellular nitrogen transport.

The GS in root plastids generates amide nitrogen for local consumption; the GS in shoot chloroplasts reassimilates photorespiratory NH4+ . Light and carbohydrate levels alter the expression of the plastid forms of the enzyme, but they have little effect on the cytosolic forms.

- Elevated plastid levels of glutamine stimulate activity of **glutamate synthase** (also known as *glutamine:2-oxoglutarate aminotransferase*, or **GOGAT**). This enzyme transfers amide group of glutamine to 2-oxoglutarate, yielding 2 molecules of glutamate.
- Plants contain 2 types of GOGAT: One accepts electrons from NADH; other accepts electrons from ferredoxin (Fd):

Glutamine + 2-oxoglutarate + NADH + H+ → 2 glutamate + NAD+ (12.4)

Glutamine + 2-oxoglutarate + Fd_{red} → 2 glutamate + Fdox (12.5)



-NADH type of enzyme (NADH-GOGAT) is located in plastids of nonphotosynthetic tissues such as roots or vascular bundles of developing leaves. In roots, NADH-GOGAT is involved in assimilation of NH_4^+ absorbed from rhizosphere (the soil near surface of roots); in vascular bundles of developing leaves, NADH-GOGAT assimilates glutamine translocated from roots or senescing leaves.

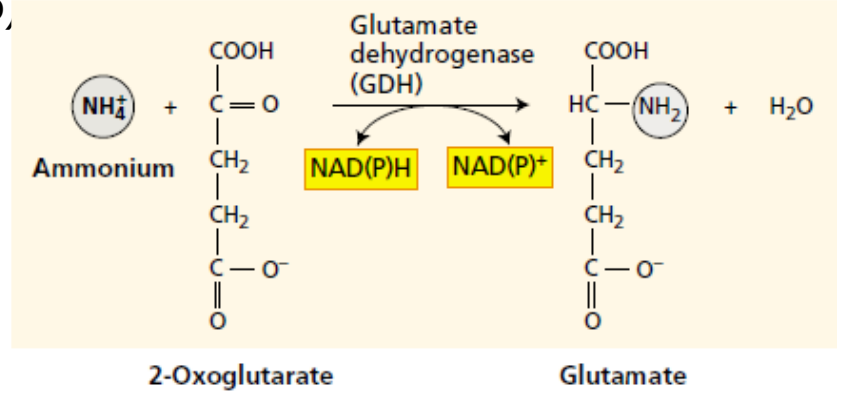
Ferredoxin-dependent type of glutamate synthase (Fd- GOGAT) is found in chloroplasts and serves in photorespiratory nitrogen metabolism. Both the amount of protein and its activity increase with light levels. Roots, particularly those under nitrate nutrition, have Fd-GOGAT in plastids. Fd-GOGAT in the roots presumably functions to incorporate the glutamine generated during nitrate assimilation.

Ammonium Can Be Assimilated via an Alternative Pathway

Glutamate dehydrogenase (**GDH**) catalyzes a *reversible* reaction that synthesizes or deaminates glutamate

2-Oxoglutarate + NH4+ + NAD(P)H -→ glutamate + H_2O + NAD(P)+ (12.6)

An NADH-dependent form of GDH is found in mitochondria, and an NADPH-dependent form is localized in the chloroplasts of photosynthetic organs. Although both forms are relatively abundant, they cannot substitute for the GS–GOGAT pathway for assimilation of ammonium, and their primary function is to deaminate glutamate.



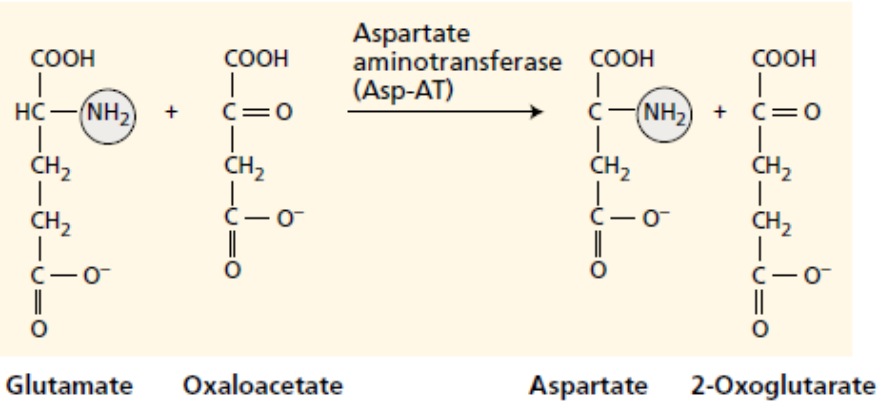
Transamination Reactions Transfer Nitrogen

Once assimilated into glutamine and glutamate, nitrogen is incorporated into other amino acids via transamination reactions. The enzymes that catalyze these reactions are known as *aminotransferases*. An example is **aspartate aminotransferase** (**Asp-AT**), which catalyzes the reaction:

Glutamate + oxaloacetate → aspartate + 2-oxoglutarate (12.7)

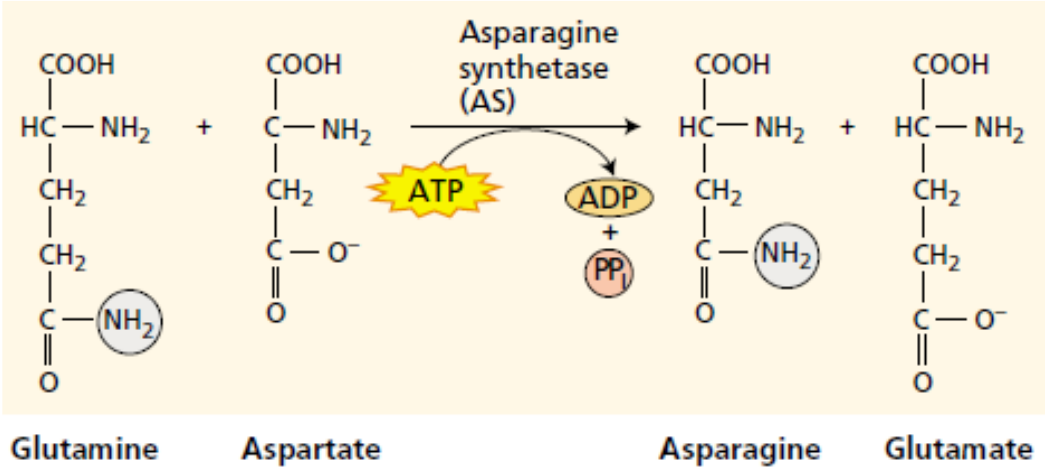
in which amino group of glutamate is transferred to the carboxyl atom of aspartate. Aspartate is an amino acid that participates in the malate–aspartate shuttle to transfer reducing equivalents from mitochondrion and chloroplast into cytosol and in transport of carbon from mesophyll to bundle sheath for C4 carbon fixation. All transamination reactions require **pyridoxal phosphate (vitamin B6) as a cofactor**.

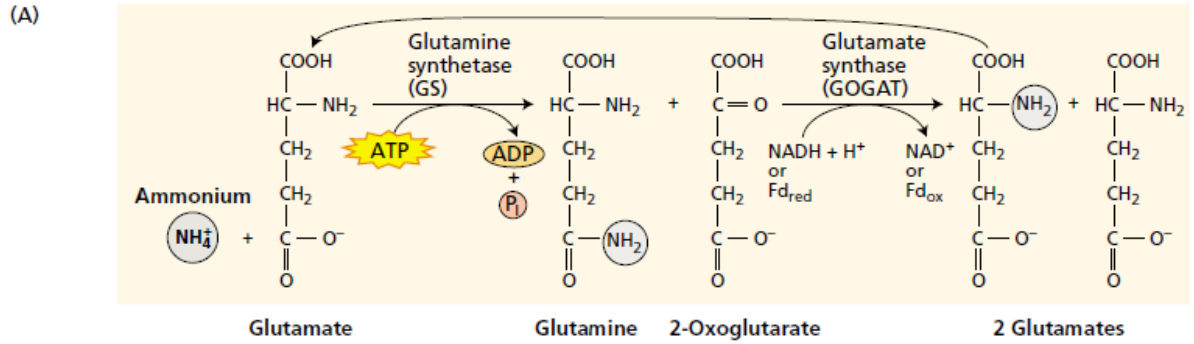
-Aminotransferases are found in cytoplasm, chloroplasts, mitochondria, glyoxysomes, and peroxisomes. The aminotransferases localized in chloroplasts may have a significant role in amino acid biosynthesis because plant leaves or isolated chloroplasts exposed to radioactively labeled CO_2 rapidly incorporate the label into glutamate, aspartate, alanine, serine, and glycine.



Asparagine and Glutamine Link Carbon and Nitrogen Metabolism

- Asparagine, isolated from asparagus as early as 1806, was the first amide to be identified.
- The major pathway for asparagine synthesis involves the transfer of the amide nitrogen from glutamine to asparagine **Glutamine + aspartate + ATP → asparagine + glutamate + AMP + PP_i** (12.8)
- **Asparagine synthetase** (**AS**), the enzyme that catalyzes this reaction, is found in the cytosol of leaves and roots and in nitrogen-fixing nodules. In maize roots, particularly those under potentially toxic levels of ammonia, ammonium may replace glutamine as the source of the amide group.
- High levels of light and carbohydrate—conditions that stimulate plastid GS and Fd-GOGAT—inhibit the expression of genes coding for AS and the activity of the enzyme.
- The opposing regulation of these competing pathways helps balance the metabolism of carbon and nitrogen in plants. Conditions of ample energy (i.e., high levels of light and carbohydrates) stimulate GS and GOGAT, inhibit AS, and thus favor nitrogen assimilation into glutamine and glutamate, compounds that are rich in carbon and participate in the synthesis of new plant materials.
- By contrast, energy-limited conditions inhibit GS and GOGAT, stimulate AS, and thus favor nitrogen assimilation into asparagine, a compound that is rich in nitrogen and sufficiently stable for long-distance transport or long-term storage.





Structure and pathways of compounds involved in ammonium metabolism. Ammonium can be assimilated by one of several processes.

(A) The GS-GOGAT pathway that forms glutamine and glutamate. A reduced cofactor is required for the reaction: ferredoxin in green leaves and NADH in nonphotosynthetic tissue.

(B) The GDH pathway that forms glutamate using NADH or NADPH as a reductant.

(C) Transfer of the amino group from glutamate to oxaloacetate to form aspartate (catalyzed by aspartate aminotransferase).

(D) Synthesis of asparagine by transfer of an amino acid group from glutamine to aspartate (catalyzed by asparagine synthesis).

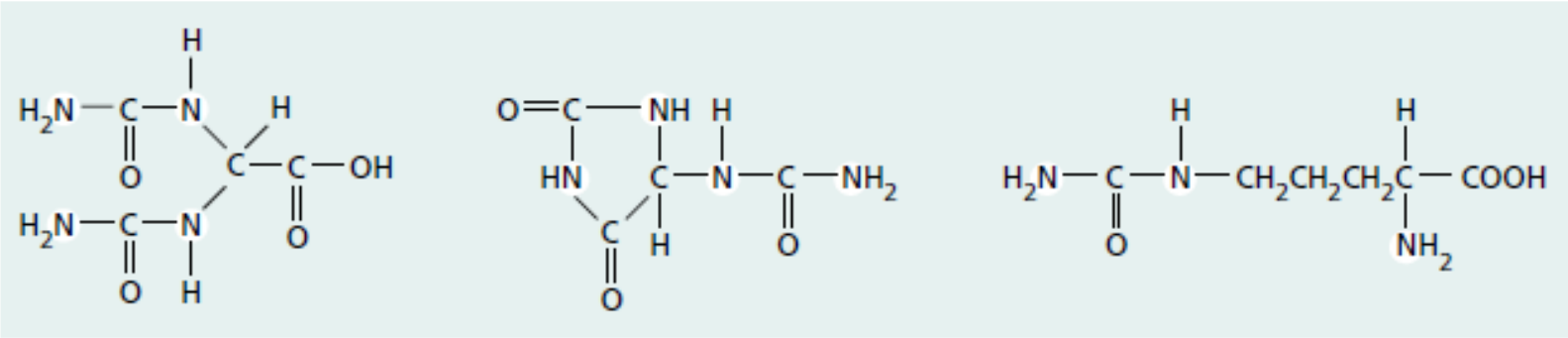
Amides and Ureides Are the Transported Forms of Nitrogen

- The symbiotic nitrogen-fixing prokaryotes release ammonia that, to avoid toxicity, must be rapidly converted into organic forms in the root nodules before being transported to the shoot via the xylem.

- **Nitrogen-fixing legumes** can be divided into **amide exporters or ureide exporters** on the basis of the composition of the xylem sap. *Amides (principally the amino acids asparagine or glutamine*) are exported by temperate-region legumes, such as pea (*Pisum*), clover (*Trifolium*), broad bean (*Vicia*), and lentil (*Lens*).

- Ureides are exported by legumes of tropical origin, such as soybean (*Glycine*), kidney bean (*Phaseolus*), peanut (*Arachis*), and southern pea (*Vigna*). The three major ureides are allantoin, allantoic acid, and citrulline. Allantoin is synthesized in peroxisomes from uric acid, and allantoic acid is synthesized from allantoin in ER.

- All three compounds are ultimately released into the xylem and transported to the shoot, where they are rapidly catabolized to ammonium. This ammonium enters the assimilation pathway described earlier.



The major ureide compounds used to transport nitrogen from sites of fixation to sites where their deamination will provide nitrogen for amino acid and nucleoside synthesis

(Note: All the original contributors of the concept and findings published elsewhere are gratefully acknowledged while preparing the E-content for the purpose of student reading material in convenient form for biochemistry and allied discipline).

Reference

- Taiz, L. and Zeiger, E. (2006) Plant physiology. 4th Edition, Sinauer Associates, Inc., Sunderland.