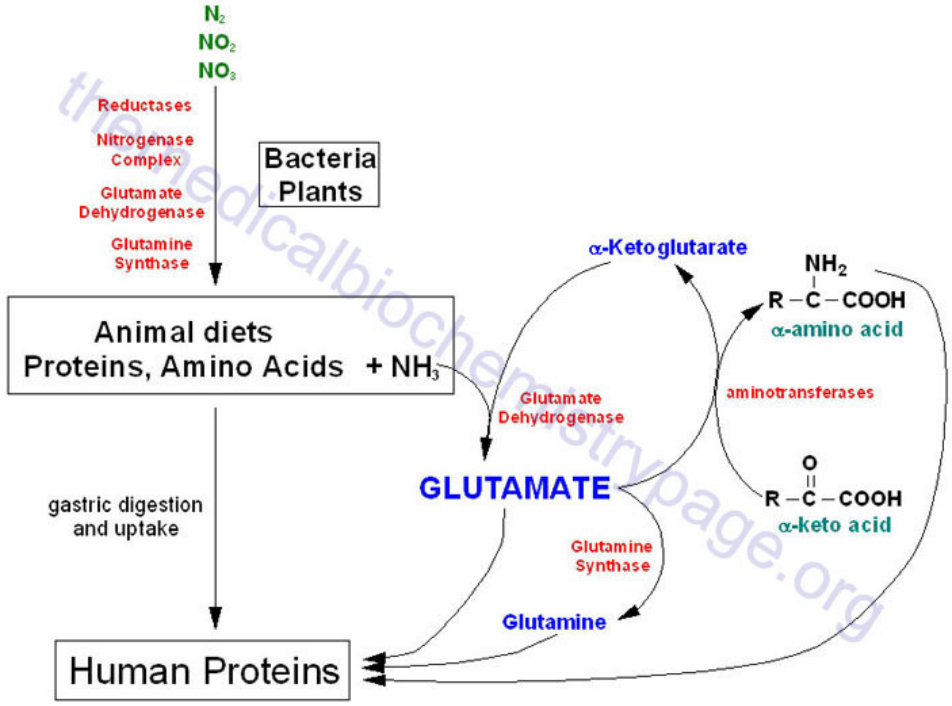
# Nitrogen Metabolism and the Urea Cycle

## Introduction

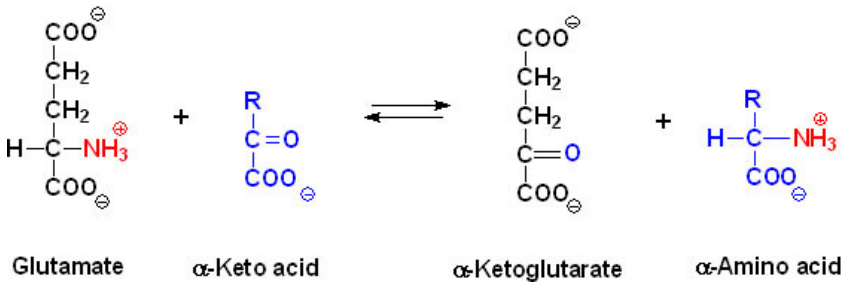
Humans are totally dependent on other organisms for converting atmospheric nitrogen into forms available to the body. Nitrogen fixation is carried out by bacterial nitrogenases forming reduced nitrogen, $NH_4^+$, which can then be used by all organisms to form amino acids.





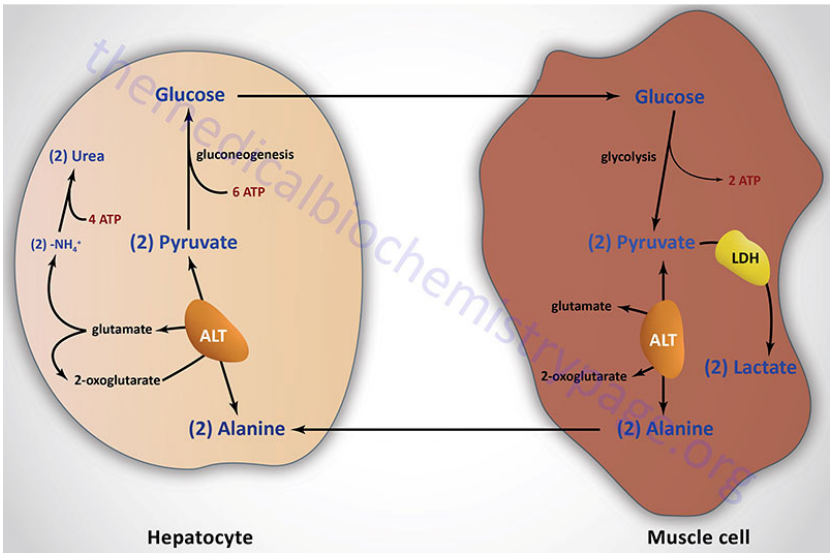

**Overview of the flow of nitrogen in the biosphere.** Nitrogen, nitrites and nitrates are acted upon by bacteria (nitrogen fixation) and plants and we assimilate these compounds as protein in our diets. Ammonia incorporation in animals occurs through the actions of glutamate dehydrogenase and glutamine synthetase. Glutamate plays the central role in mammalian nitrogen flow, serving as both a nitrogen donor and nitrogen acceptor.

Reduced nitrogen enters the human body as dietary free amino acids, protein, and the ammonia produced by intestinal tract bacteria. A pair of principal enzymes, glutamate dehydrogenase and glutamine synthetase, are found in all organisms and effect the conversion of ammonia into the amino acids glutamate and glutamine, respectively. Amino and amide groups from these 2 substances are freely transferred to other carbon skeletons by transamination and transamidation reactions.



## Representative aminotransferase catalyzed reaction.

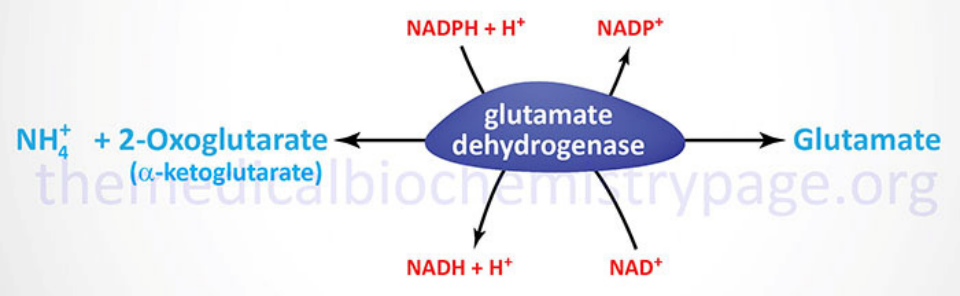
Aminotransferases (transaminases) catalyze the general reaction shown above. The most common compounds involved as a donor/acceptor pairs in transamination reactions are glutamate and 2-oxoglutarate (α-ketoglutarate). Alanine transaminase has an important function in the delivery of skeletal muscle carbon and nitrogen (in the form of alanine) to the liver. In skeletal muscle, pyruvate is transaminated to alanine, thus affording an additional route of nitrogen transport from muscle to liver. In the liver, alanine transaminase transfers the ammonia to 2-oxoglutarate and regenerates pyruvate. The pyruvate can then be diverted into gluconeogenesis forming new glucose for release to the blood. This process is referred to as the glucose-alanine cycle.




**The glucose-alanine cycle:** The glucose-alanine cycle is used primarily as a mechanism for skeletal muscle to eliminate nitrogen while replenishing its energy supply. Glucose oxidation produces pyruvate which can undergo transamination to alanine. This reaction is catalyzed by alanine transaminase (ALT) which is encoded by the GPT (glutamate-pyruvate transaminase) gene. Additionally, during periods of fasting, skeletal muscle protein is degraded for the energy value of the amino acid carbons and alanine is a major amino acid in protein. The alanine then enters the blood stream and is transported to the liver. Within the liver alanine is converted back to pyruvate which is then a source of carbon atoms for gluconeogenesis. The newly formed glucose can then enter the blood for delivery back to the muscle. The amino group transported from the muscle to the liver in the form of alanine is converted to urea in the urea cycle and excreted.

# The Glutamate Dehydrogenase Reaction

The reaction catalyzed by glutamate dehydrogenase is:




Glutamate dehydrogenase (GDH) utilizes both nicotinamide nucleotide cofactors; $NAD^+$ in the direction of nitrogen liberation and NADPH for nitrogen incorporation. In the forward reaction (as shown above) glutamate dehydrogenase is important in converting free ammonia (as ammonium ion, $NH_4^+$) and 2-oxoglutarate (α-ketoglutarate) to glutamate, forming one of the 20 amino acids required for protein synthesis while simultaneously reducing the cellular load of potentially toxic ammonium ion. However, it should be recognized that the reverse reaction is a key anapleurotic process linking amino acid metabolism with TCA cycle activity. In the reverse reaction, glutamate dehydrogenase provides an oxidizable carbon source used for the production of energy as well as a reduced electron carrier, NADH. As expected for a branch point enzyme with an important link to energy metabolism, glutamate dehydrogenase is regulated by the cell energy charge. ATP and GTP are positive allosteric effectors of the formation of glutamate, and conversely, ATP and GTP exert potent negative allosteric effects on the formation of 2-oxoglutarate. GTP is unique in its inhibitory effects on GDH, compared to ATP, in that the inhibition by GTP affects all of the subunits of the homohexameric complex regardless of the subunit to which GTP is bound. This effect of GTP makes this nucleotide the more potent regulator of GDH activity. NADH is also an allosteric inhibitor of the 2-oxoglutarate liberating direction of the GDH reaction. Given that low energy charge would be expected to increase the conversion of glutamate to 2-oxoglutarate, it is not surprising that ADP is a positive allosteric effector of this reaction direction. Thus, when the level of ATP is high, conversion of glutamate to 2-oxoglutarate and other TCA cycle intermediates is limited; when the cellular energy charge is low, glutamate is converted to ammonia and oxidizable TCA cycle intermediates. Glutamate is also a principal amino donor to other amino acids in subsequent transamination reactions. The multiple roles of glutamate in nitrogen balance make it a gateway between free ammonia and the amino groups of most amino acids.

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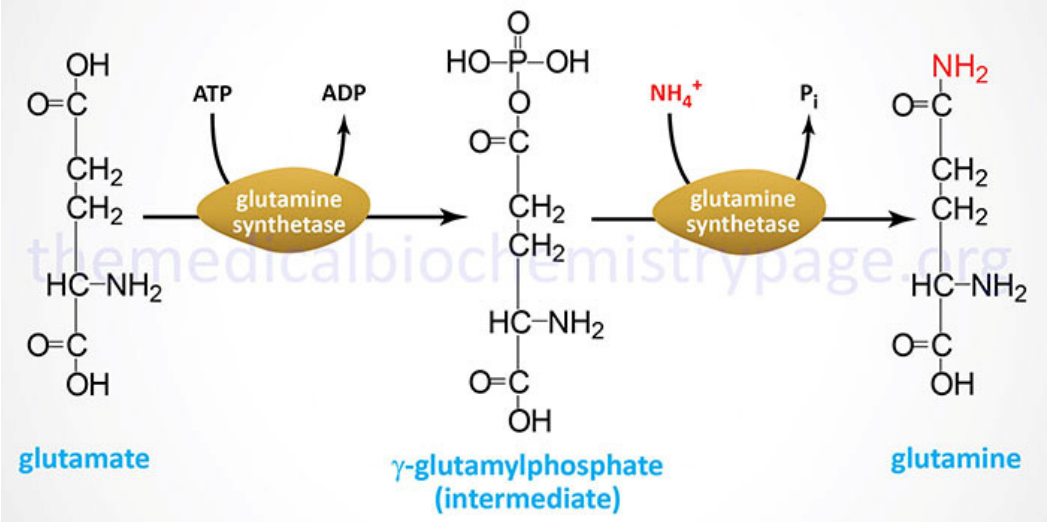
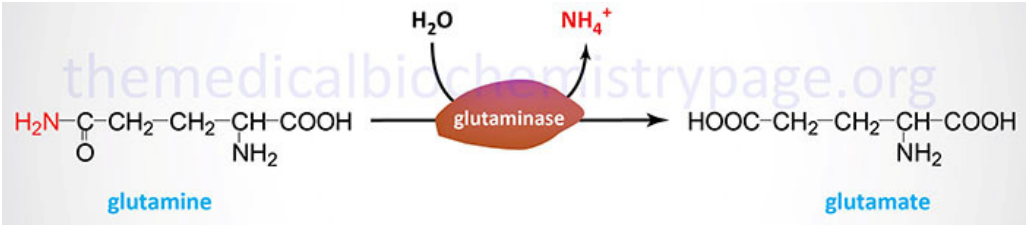
# The Glutamine Synthetase and Glutaminase Reactions

The reaction catalyzed by glutamine synthetase is:




The glutamine synthetase reaction is also important in several respects. First it produces glutamine, one of the 20 major amino acids. Second, in animals, glutamine is the major amino acid found in the circulatory system. Its role there is to carry ammonia to and from various tissues but principally from peripheral tissues to the kidney, where the amide nitrogen is hydrolyzed by the enzyme glutaminase (reaction below); this process regenerates glutamate and free ammonium ion, which is excreted in the urine.




Note that, in this function, ammonia arising in peripheral tissues is carried in a non-ionizable form which has none of the neurotoxic or alkalosis-generating properties of free ammonia.

The primary tissues that express glutaminase are the brain and kidney. In the brain the role of glutaminase is in the synthesis of the neurotransmitter glutamate. In the kidneys the role of glutaminase is in acid-base balance as discussed below. However, the liver also expresses both glutamine synthetase and glutaminase but the enzymes are localized in different subsets of hepatocytes. This ensures that the liver is neither a net producer nor consumer of glutamine. The differences in cell location of these two enzymes allows the liver to scavenge ammonia that has not been incorporated into urea. The enzymes of the urea cycle are located in the same cells as those that contain glutaminase. The result of the differential distribution of these two hepatic enzymes makes it possible to control ammonia incorporation into either urea or glutamine, the latter leads to excretion of ammonia by the kidney.

When acidosis occurs the body will divert more glutamine from the liver to the kidney. This allows for the conservation of bicarbonate ion since the incorporation of ammonia into urea requires bicarbonate (see below). When glutamine enters the kidney, glutaminase releases one mole of ammonia generating glutamate and then glutamate dehydrogenase releases another mole of ammonia generating 2-oxoglutarate (α-ketoglutarate). The ammonia will ionize to ammonium ion ($NH_4^+$) which is excreted. The net effect is a reduction in the concentration of hydrogen ion,

[$H^+$], and thus an increase in the pH (see also Renal Acid-Base Balance). This process is referred to as proximal tubular ammoniagenesis.

The glutamine synthetase enzyme is encoded by the glutamate-ammonia ligase gene (symbol: GLUL) which is located on chromosome 1q25.3 and is composed of 9 exons that generate three alternatively spliced mRNAs, all of which encode the same 373 amino acid protein. There are two distinct glutaminase genes in humans identified as GLS (encoding the GLS1 enzyme) and GLS2 (encoding the GLS2 enzyme). The GLS gene is located on chromosome 2q32.2 and is composed of 20 exons that undergo alternative splicing to yield two mRNAs generating two isoforms of the enzyme. These two GLS-derived isoforms are often referred to as glutaminase C (GAC) and kidney-type glutaminase (KGA) but are collectively the glutaminase 1 (GLS1) enzymes. The GLS encoded isoforms of glutaminase are primarily expressed in the kidneys. GLS encoded kidney-type glutaminase is a protein of 669 amino acids and GLS encoded glutaminase C is a protein of 598 amino acids. The GLS2 gene encoded glutaminase was originally thought to be liver specific but is in fact expressed in numerous tissues and is important in the glutamate-glutamine cycle in the brain. The GLS2 encoded glutaminase was originally characterized as dependent on inorganic phosphate ($P_i$) for activity and is, therefore, also referred to as phosphate-activated glutaminase, PAG. However, both the GLS gene encoded enzymes and the GLS2 encoded enzymes require phosphate for activity with GLS enzymes being more sensitive. The GLS2 gene is located on chromosome 12q13.3 and is composed of 19 exons that undergo alternative splicing to yield four mRNAs that encode four different isoforms of the enzyme. The GLS encoded enzymes are inhibited by glutamate but the GLS2 encoded enzyme is not. The GLS2 encoded enzyme is activated by ammonia but the GLS encoded enzymes are not.

## Essential vs. Nonessential Amino Acids

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|---|---|
| **Nonessential** | Alanine, Asparagine, Aspartate, Cysteine, Glutamate, Glutamine, Glycine, Proline, Serine, Tyrosine |
| **Essential** | Arginine*, Histidine, Isoleucine, Leucine, Lysine, Methionine*, Phenylalanine*, Threonine, Tryptophan, Valine |

*The amino acids **arginine**, **methionine** and **phenylalanine** are considered essential for reasons not directly related to lack of synthesis. Arginine is synthesized by mammalian cells but at a rate that is insufficient to meet the growth needs of the body and the majority that is synthesized is cleaved to form urea. Methionine is required in large amounts to produce cysteine if the latter amino acid is not adequately supplied in the diet. Similarly, phenylalanine is needed in large amounts to form tyrosine if the latter is not adequately supplied in the diet.

## Removal of Nitrogen from Amino Acids

The dominant reactions involved in removing amino acid nitrogen from the body are known as transaminations (see general reaction above). This class of reactions funnels nitrogen from all free amino acids into a small number of compounds; then, either they are oxidatively deaminated, producing ammonia, or their amine groups are converted to urea by the urea cycle. Transaminations involve moving an α-amino group from a donor α-amino acid to the keto carbon of an acceptor α-keto acid. These reversible reactions are catalyzed by a group of intracellular enzymes known

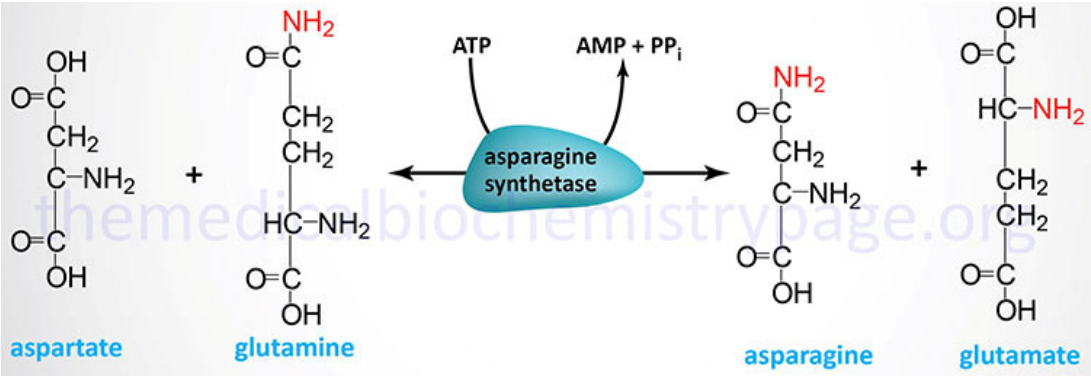
as aminotransferases (also called transaminases), most all of which require covalently bound pyridoxal phosphate (vitamin $B_6$) as a cofactor. However, some aminotransferases employ pyruvate as a cofactor.

Aminotransferases (transaminases) exist for all amino acids except threonine and lysine. The most common compounds involved as a donor/acceptor pairs in transamination reactions are glutamate and 2-oxoglutarate (α-ketoglutarate), which participate in reactions with many different aminotransferases. Serum aminotransferases such as aspartate aminotransferase, AST (also called serum glutamate-oxaloacetate transaminase, SGOT) and alanine transaminase, ALT (also called serum glutamate-pyruvate transaminase (SGPT) have been used as clinical markers of tissue damage, with increasing serum levels indicating an increased extent of damage (see the Enzyme Kinetics page for description of the use of enzyme levels in diagnosis). As indicated earlier, ALT has an important function in the delivery of skeletal muscle carbon and nitrogen (in the form of alanine) to the liver in a series of reactoins referred to as the glucoase-alanine cycle (see Figure above in Introduction section). In skeletal muscle, pyruvate is transaminated to alanine, thus affording an additional route of nitrogen transport from muscle to liver. In the liver, alanine transaminase transfers the ammonia to 2-oxoglutarate and regenerates pyruvate. The pyruvate can then be diverted into gluconeogenesis allowing the liver to generate endogenous glucose which it can then release to the blood where skeletal muscle can use it for anaerobic energy production.

ALT is a cytosolic enzyme encoded by the GPT (glutamate-pyruvate transaminase) gene which is located on chromosome 8q24.3 and is composed of 12 exons that encode a 496 amino acid protein. Humans express two different AST enzymes, both of which function as homodimeric enzymes. One AST enzyme is a cytosolic enzyme and the other is a mitochondrial enzyme. The cytosolic AST enzyme is synthesized by the GOT1 gene (glutamate-oxalate transaminase 1) that is located on chromosome 10q24.2 and is composed of 9 exons that encode a 413 amino acid protein. The mitochondrial AST enzyme is synthesized from the GOT2 gene that is located on chromosome 16q21 and is composed of 10 exons that generate two alternatively spliced mRNAs that encode two different isoforms: isoform 1 (430 amino acids) and isoform 2 (387 amino acids).

Because of the participation of 2-oxoglutarate in numerous transaminations, glutamate is a prominent intermediate in nitrogen elimination as well as in anabolic pathways. Glutamate, formed in the course of nitrogen elimination, is either oxidatively deaminated by liver glutamate dehydrogenase forming ammonia which is then incorporated into urea, or converted to glutamine by glutamine synthetase and transported to proximal tubule cells in the kidney. There the glutamine is sequentially deamidated by glutaminase and deaminated by kidney glutamate dehydrogenase releasing $NH_3$ which ionizes with $H^+$ forming ammonium ion ($NH_4^+$). The $NH_4^+$ is excreted in the urine, where it helps increase serum pH in conditions of metabolic acidosis as well as being involved in the maintenance of urine pH in the normal range of pH 4 to pH 8. The extensive production of ammonia by peripheral tissue or hepatic glutamate dehydrogenase is not feasible because of the highly toxic effects of circulating ammonia. Normal serum ammonium concentrations are in the range of 20–40μM, and an increase in circulating ammonia to about 400μM causes alkalosis and neurotoxicity.

A final, therapeutically useful amino acid-related reaction is the amidation of aspartic acid to produce asparagine. The enzyme, asparagine synthetase (gene symbol: ASNS), catalyzes the ATP-requiring transamidation reaction shown below. The ASNS gene is located on chromosome 7q21.3 and is composed of 15 exons that generate seven alternatively spliced mRNAs that collectively encode three distinct protein isoforms.




Most cells perform this reaction well enough to produce all the asparagine they need. However, some leukemia cells require exogenous asparagine, which they obtain from the plasma. Chemotherapy using the enzyme asparaginase takes advantage of this property of leukemic cells by hydrolyzing serum asparagine to ammonia and aspartic acid, thus depriving the neoplastic cells of the asparagine that is essential for their characteristic rapid growth.

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# The Urea Cycle

About 80% of the excreted waste nitrogen is in the form of urea which is produced exclusively in the liver, in a series of reactions that are distributed between the mitochondrial matrix and the cytosol. The series of reactions that form urea is known as the Urea Cycle or the Krebs-Henseleit Cycle.

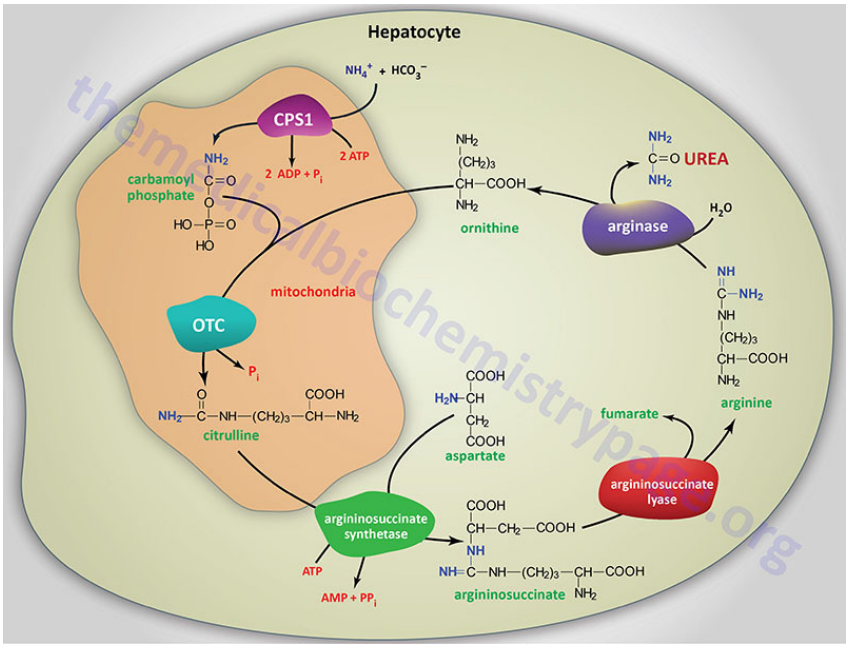

**Diagram of the urea cycle.** The reactions of the urea cycle which occur in the mitochondrion are contained in the red rectangle. All enzymes are in red, CPS1 is carbamoyl phosphate synthetase-I, OTC is ornithine transcarbamoylase. Click on the enzyme names to go to a descriptive page of the urea cycle disorder caused by deficiency in the particular enzyme.

The essential features of the urea cycle reactions are that free ammonium ion, generated from the glutaminase and glutamate dehydrogenase reactions, is condensed with bicabonate and eventually converted to urea for excretion. In addition to the arginine produced in the urea cycle, arginine from the diet or from protein breakdown can be cleaved by the cytosolic enzyme arginase, generating urea and ornithine. Ornithine, arising in the cytosol, is transported to the mitochondrial matrix via the action of ornithine translocase encoded by the ORNT1 gene. The ORNT1 transporter is a member of the solute carrier family of transporters and as such is also identified as SLC25A15. In subsequent reactions of the urea cycle a new urea residue is built on the ornithine from additional ammonium ions, regenerating arginine and perpetuating the cycle. Concomitant with ornithine transport into the mitochondria is the export of citrulline, by SLC25A15, to the cytosol where the remaining reactions of the cycle take place. Also important in the function of the urea cycle is the mitochodrial transporter called citrin. Citrin is involved in the mitochondrial uptake of glutamate and export of aspartate and as such functions, in part, in the malate-aspartate shuttle. Citrin is a $Ca^{2+}$-dependent mitochondrial solute transporter that is also a member of the solute carrier family of transporters identified as SLC25A13.

In the mitochondria ornithine transcabamoylase (OTC) catalyzes the condensation of ornithine with carbamoyl phosphate, producing citrulline. The OTC gene is an X-linked gene (Xp11.4) composed of 10 exons that encode a precursor protein of 354 amino acids. The energy for the OTC reaction is provided by the high-energy anhydride of carbamoyl phosphate. The synthesis of citrulline requires a prior activation of carbon and nitrogen as carbamoyl phosphate (CP). The activation step requires two equivalents of ATP and the mitochondrial matrix enzyme carbamoyl phosphate synthetase 1(CPS1 or CPS-I). The reaction catalyzed by CPS1 is the rate-limiting reaction of the urea cycle. The CPS1 enzyme is encoded by the CPS1 gene which is located on chromosome 2q34 and is composed of 43 exons that generate three alternatively spliced mRNAs. These three mRNAs generate three isoforms of CPS1: isoform a is a protein of 1506 amino acids, isoform b is a protein of 1500 amino acids, and isoform c is a protein of 1049 amino acids. The catalytic activity of CPS1 is positively regulated by *N*-acetylglutamate which is produced by *N*-acetylglutamate synthetase (NAGS). In the absence of *N*-acetylglutamate there is little, if any, CPS1 activity such that this molecule is often referred to as an obligate activator as opposed to an allosteric activator. The NAGS gene is located on chromosome 17q21.31 and is composed of 7 exons that encode a 534 amino acid protein.

There are two carbamoyl phosphate synthetase activities in human cells: the mitochondrial CPS1 which forms CP destined for inclusion in the urea cycle, and a cytosolic carbamoyl synthetase activity (the CPS2 activity of a tri-functional enzyme complex), which is involved in pyrimidine nucleotide biosynthesis. The cytosolic enzyme (encoded by the CAD gene) that possesses the CPS2 (also designated CPS-II) activity is not affected by *N*-acetylglutamate.

In a 2-step reaction, catalyzed by cytosolic argininosuccinate synthetase, citrulline and aspartate are condensed to form argininosuccinate. The reaction involves the addition of AMP (from ATP) to the amido carbonyl of citrulline, forming

an activated intermediate on the enzyme surface (AMP-citrulline), and the subsequent addition of aspartate to form argininosuccinate. Argininosuccinate synthetase is encoded by the ASS1 gene located on chromosome 9q34.11 which is composed of 18 exons that generate two alternatively spliced mRNAs that generate the same 412 amino acid protein. The human genome contains at least 14 copies of the ASS1 gene all of which are pseudogenes except the one on chromosome 9 which encodes the functional enzyme.

Arginine and fumarate are produced from argininosuccinate by the cytosolic enzyme argininosuccinate lyase (also called argininosuccinase). Argininosuccinate lyase is functional as a homotetrameric complex. The argininosuccinate lyase protein is encoded by the ASL gene located on chromosome 7q11.21 and is composed of 17 exons that generate four alternatively spliced mRNAs that encode three distinct protein isoforms.

In the final step of the cycle arginase cleaves urea from arginine, regenerating cytosolic ornithine, which can be transported to the mitochondrial matrix for another round of urea synthesis. The fumarate, generated via the action of arginiosuccinate lyase, is reconverted to aspartate for use in the argininosuccinate synthetase reaction. This occurs through the actions of cytosolic versions of the TCA cycle enzymes, fumarase (which yields malate) and malate dehydrogenase (which yields oxaloacetate). The oxaloacetate is then transaminated to aspartate by AST. There are two arginase genes in humans identified as the ARG1 and ARG2 genes. The ARG1 encoded isoform of arginase is a cytosolic enzyme primarily expressed in the liver and functions as the urea cycle enzyme. The ARG1 gene is located on chromosome 6q23.2 and is composed of 8 exons that generate two alternatively spliced mRNAs encoding arginase-1 isoform 1 (330 amino acids) and arginase-1 isoform 2 (322 amino acids). The ARG2 encoded arginase (arginase-2) is localized to the mitochondria in non-hepatic tissues, primarily the kidney. The arginase-2 isoform is thought to be involved in nitric oxide and polyamine metabolism, however, the precise role of this enzyme is not clearly defined. The ARG2 gene is located on chromosome 14q24.1 and is composed of 8 exons that encode a precursor protein of 354 amino acids.

Beginning and ending with ornithine, the reactions of the cycle consume three equivalents of ATP and a total of four high-energy nucleotide phosphates. Urea is the only new compound generated by the cycle; all other intermediates and reactants are recycled. The energy consumed in the production of urea is more than recovered by the release of energy formed during the synthesis of the urea cycle intermediates. Ammonia released during the glutamate dehydrogenase reaction is coupled to the formation of NADH. In addition, when fumarate is converted back to aspartate, the malate dehydrogenase reaction used to convert malate to oxaloacetate generates a mole of NADH. These two moles of NADH are subsequently oxidized in the mitochondria yielding six moles of ATP.

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## Regulation of the Urea Cycle

The urea cycle operates only to eliminate excess nitrogen. On high-protein diets the carbon skeletons of the amino acids are oxidized for energy or stored as fat and glycogen, but the amino nitrogen must be excreted. To facilitate this process, enzymes of the urea cycle are controlled at the gene level. With long-term changes in the quantity of dietary protein, changes of 20-fold or greater in the concentration of cycle enzymes are observed. When dietary proteins increase significantly, enzyme concentrations rise. On return to a balanced diet, enzyme levels decline. Under conditions of starvation, enzyme levels rise as proteins are degraded and amino acid

carbon skeletons are used to provide energy, thus increasing the quantity of nitrogen that must be excreted.

Short-term regulation of the cycle occurs principally at the CPS1 reaction which is the rate-limiting reaction of the urea cycle. CPS1 is essentially inactive in the absence of the activator *N*-acetylglutamate such that this molecule is sometimes referred to as an obligate activator as opposed to an allosteric activator. The steady-state concentration of *N*-acetylglutamate is set by the cellular concentrations of acetyl-CoA and glutamate which are used by the enzyme *N*-acetylglutamate synthase (NAGS) to form *N*-acetylglutamate. The NAGS gene is located on chromosome 17q21.31 and is composed of 7 exons that encode a mitochondrial protein of 534 amino acids. The activity of NAGS is allosterically activated by the amino acid and urea cycle intermediate, arginine. Indeed, the allosteric activation of NAGS by arginine explains the theerapeutic benefit of adding arginine to the diet of patients with urea cycle disorders. The increased NAGS activity will result in enhanced CPS1 activity resulting in enhanced incorporation of ammonia into the less toxic intermediate, carbamoyl phosphate.