Amino Acid Homeostasis in Mammalian Cells with a Focus on Amino Acid Transport

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ABSTRACT
Amino acid homeostasis is maintained by import, export, oxidation, and synthesis of nonessential amino acids, and by the synthesis and breakdown of protein. These processes work in conjunction with regulatory elements that sense amino acids or their metabolites. During and after nutrient intake, amino acid homeostasis is dominated by autoregulatory processes such as transport and oxidation of excess amino acids. Amino acid deprivation triggers processes such as autophagy and the execution of broader transcriptional programs to maintain plasma amino acid concentrations. Amino acid transport plays a crucial role in the absorption of amino acids in the intestine, the distribution of amino acids across cells and organs, the recycling of amino acids in the kidney, and the recycling of amino acids after protein breakdown. J Nutr 2021;00:1–13.

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Introduction
Homeostasis is one of the fundamental concepts in physiology. The concept was initially developed by Claude Bernard after observing that the physical and chemical properties of the “milieu interieur” remained largely unaffected by environmental changes. Bernard posited that constant internal conditions liberated animals from the dynamic changes of the environment (1). The concept was then further developed by Haldane, Henderson, and Cannon (2) and remains an overarching theme in physiology (3).

Like many other metabolites, amino acid concentrations are kept within narrow limits. Accordingly, standard amino acid concentrations are routinely used to identify rare disorders in which amino acid concentrations deviate significantly from normal amino acid concentrations in plasma or urine (4). Smaller deviations can also be indicative of disease states such as diabetes (5). Many inherited disorders of amino acid metabolism are associated with neurological symptoms due to disturbances of amino acid homeostasis in the brain, where it is most critical (6).

Fundamentally, there are 6 contributors to amino acid homeostasis in mammalian cells, namely 1) import, 2) export, 3) metabolism, and 4) synthesis of nonessential amino acids, 5) protein synthesis, and 6) protein breakdown (Figure 1). These processes work in conjunction with regulatory elements that respond to amino acids or their metabolites. In the following, these contributors will be discussed to assess their role in amino acid homeostasis.

Export and Import
The small intestine is the main site of organismic amino acid absorption in the form of individual amino acids, di-, and tripeptides (7). Digestion of proteins is an efficient process ranging from 97% digestibility of crude protein in eggs to ≥70% in cereal (8). Protein absorption is essentially complete at the end of the ileum, leaving only trace amounts of amino acids in fecal matter (8, 9). Little absorption occurs in the colon (10, 11). Figure 2 shows an overview of amino acid

[Figure 2: An overview of amino acid transport processes in mammals]

[Figure 1: Contributors to amino acid homeostasis]
absorption in the small intestine. Di- and tripeptides are absorbed by the intestinal peptide transporter PepT1 (Peptide transporter 1, SLC15A1) in the apical membrane of enterocytes (12). Complete removal is achieved by coupling the peptide uptake with the cotransport of protons, which have a large electrochemical gradient in the intestine owing to a low luminal pH of 6.0 and a membrane potential of $-30$ mV (13). Only very small amounts of peptides pass through enterocytes due to efficient hydrolysis in the cytosol (14). Because of the close association between membrane-embedded peptidases and transporters (15, 16), it is difficult to estimate the proportion of digested protein that is absorbed as individual amino acids or peptides, but both pathways appear to be of similar importance.

Concentrative amino acid transporters (Figure 2) are expressed on the apical membrane for the absorption of neutral amino acids (B0AT1, broad neutral amino acid transporter 1, SLC6A19) (17, 18), cationic amino acids (b0,AT, blastocyst neutral and cationic amino acid transporter, SLC7A9) (19, 20), glycine and proline (PAT1, proton amino acid transporter 1, SLC36A1; SIT, system imino transporter, SLC6A20) (21, 22), anionic amino acids [excitatory amino acid transporter 3 (EAAT3), SLC1A1] (23), and β-amino acids (PAT1, SLC36A1, TauT, taurine transporter, SLC6A6) (21, 24). B0AT1 is a Na+-amino acid cotransporter using the electrochemical gradient of Na+ to drive absorption (25–27). Although there is competition between its 16 substrates, expression levels are sufficient to absorb all substrates even at high protein loads (28). In the intestine, trafficking of B0AT1 to the apical membrane is mediated by the brush-border peptidase angiotensin-converting enzyme 2 (ACE2) (16, 29), with which it forms a dimer of heterodimers (30). A complex of similar architecture is formed by rBAT-b0,AT (31). The absorption of cationic amino acids via b0,AT is driven by the efflux of neutral amino acids and the membrane potential favoring cation import (32, 33). Neutral amino acids are recaptured by B0AT1 in enterocytes located further downstream. Glycine and proline, both of which have low affinity for B0AT1, have additional transporters, namely the proton-amino acid transporter PAT1 (21) and the 2Na+/Cl−/taurine symporter SIT1 (22, 34). PAT1 also contributes to the uptake of β-amino acids such as taurine and β-alanine (35), which are otherwise absorbed by the 2Na+/Cl−/taurine transporter TauT (24). SIT1 and TauT use the electrochemical driving force of 2Na+/Cl− to accumulate their substrates in the cytosol (36, 37). The strong vectorial activity of apical transporters ensures almost complete removal of amino acids from the lumen of the intestine. It is noteworthy that even an empty intestine will contain small amounts of amino acids, owing to shedding of cells, bacterial and enzymatic digestion of mucins, defensins, and other proteins. Loss of these proteins in fecal matter or by microbial metabolism is one of the inevitable losses (route 2 in Figure 1) of amino acids, which in humans amount to $\sim 10$ g/d (38).

Amino acid release across the basolateral membrane is mediated by a separate set of transporters (Figure 2). Neutral amino acids are released through a combination of LAT2 [large
neutral amino acid transporter 2 (39–42), LAT4 (43), and TAT1 [T-type amino acid transporter 1 (44, 45)]. Knockout studies suggest that any of these transporters are redundant individually (46, 47), but a combination could severely affect amino acid absorption. TAT1 (45) and LAT4 (48) are unisporters mediating facilitated diffusion of aromatic and branched-chain amino acids (BCAAs). LAT2 is an antiporter that accepts all neutral amino acids except proline (41). As a result it can aid in the efflux of amino acids not covered by LAT4 and TAT1 (44, 49). The antiporter y⁺LAT1 (cationic and large neutral amino acid transporter 1) is designed to facilitate the efflux of cationic amino acids in exchange for neutral amino acids plus Na⁺ (50–52). Owing to the prevalence of sodium ions in blood plasma, uptake of neutral amino acids via y⁺LAT1 is in cotransport with Na⁺. LAT2 and y⁺LAT1 form complexes with the ancillary protein 4F2hc which are very similar to the complex formed between LAT1 and 4F2hc (53). No efflux pathway for anionic amino acids has been identified, but enterocytes metabolize the bulk of glutamate to carbon dioxide and lactate, whereas the nitrogen is largely transferred onto alanine (54, 55). Efflux across the basolateral membrane is largely passive and indirectly driven by vectorial transport across the apical membrane. In fact, the basolateral membrane contains low expression levels of amino acid–Na⁺ symporters, such as SNAT2 (SLC38A2), which import nutrients from blood plasma during fasting, particularly glutamine (54).

An important element of organismic amino acid homeostasis is the glomerular filtration/reabsorption mechanism occurring in the kidney cortex (56–58). Glomerular filtration generates an ultrafiltrate of blood plasma, which during passage through the proximal tubule will be cleared of all amino acids and small proteins (58). One of the primary roles of the kidney is the elimination of urea, generated from metabolism of excess amino acids. As discussed below, amino acid metabolism is tightly regulated, but never ceases altogether, resulting in unavoidable losses of 17 g/d amino acid equivalent as urea, creatinine, and ammonia. The amount of urea increases as the protein component increases beyond essential replacement or when amino acids are used for gluconeogenesis. This, together with fecal losses of 10 g and small losses due to shedding of skin and hair, results in the minimum requirement of ∼30 g protein/d to replace unavoidable loss of amino acids. As a result, an important aspect of amino acid homeostasis is the efficient recycling of protein amino acids through complex breakdown via endocytosis, autophagy, and the proteasome (59). At a steady state, protein synthesis is matched by an equivalent amount of protein breakdown (60). In the postabsorptive phase net protein synthesis is observed, whereas during fasting net
protein breakdown occurs. Tubular reabsorption is part of this extensive recycling of amino acids. It is mediated by almost the same set of transporters as found in the intestine, with the exception of PAT2 (61) and PepT2 (62) replacing PAT1 and PepT1, respectively (Figure 2).

After absorption of amino acids following protein digestion, the corresponding rise of plasma amino acids is translated into a corresponding rise of cellular amino acid pools. This is mediated by a combination of secondary active transporters such as Na\(^+\)-symporters, antiporters, and uniporters (63, 64). The system is more readily understood using functional transporter definitions (Figure 3) (64). Each cell has transporters that load amino acids into the cell (loaders). Examples are SNAT1 and SNAT2 [sodium neutral amino acid transporters (63, 65–67)]. Using the electrochemical gradient of Na\(^+\), these transporters accumulate a group of amino acids against a concentration gradient. In the case of SNAT1/2, these are small and/or polar neutral amino acids (68–72), such as glutamine, alanine, serine, asparagine, and cysteine. Once inside the cell, the accumulated amino acids serve as exchange substrates to import other amino acids that do not have a loader (tertiary active transport). These are many of the essential amino acids such as BCAAs and aromatic amino acids. For example, SNAT1/2 can import glutamine, which can be used as an exchange substrate to bring in BCAAs via antiporters (harmonizers). SNAT1 (73) and ASCT2 [alanine-serine-cysteine transporters (74–76)] exchange small and medium neutral amino acids, whereas LAT1 exchanges large neutral amino acids (77–79). The harmonizing action can be illustrated by assuming that one amino acid is depleted in the cytosol. In this case, the depleted amino acid will enter the cell in exchange for an amino acid that is in abundance. As the deficient amino acid accumulates, it eventually becomes an exchange substrate itself, thereby reaching a steady-state equilibrium. This process has the effect of harmonizing the concentrations of all participating amino acids. Cationic amino acids can enter cells through loaders, which exploit the membrane potential to accumulate cations (80). These work in conjunction with harmonizers, such as y\(^+\)LAT2, which exchanges large neutral amino acids for cationic amino acids (50, 64). Notably, transporters for glutamate and aspartate are missing in Figure 3. Most cells generate glutamate from glutamine and aspartate from oxaloacetate and as a result do not require specific loaders. An exception is the brain, where glutamate serves as a neurotransmitter and is actively cleared from the synapse by EAAT1–4 [Excitatory amino acid transporters (81)]. EAATs are found outside the nervous system, but only scarcely. Figure 3 indicates the presence of controllers that can release amino acids. The function of controllers is to counteract the accumulative power of the loaders. The sodium electrochemical gradient allows an ~100-fold accumulation of substrates by SNAT1/2. Because harmonizers are tied to loaders via exchange [tertiary active transport (82)], eventually all amino acids would reach a 100-fold accumulation. The combined plasma concentration of all amino acids is ~3 mM, which would generate an osmotic load of 300 mM—doubling the normal osmolarity—and cause cell swelling. Not surprisingly, loaders are regulated by osmolarity (83). Controllers typically have low affinity for their substrates (in the mM range) and only become functionally relevant as intracellular amino acid concentrations rise. Owing to the
combined action of secondary active transporters, cytosolic amino acid concentrations are 2- to 30-fold above plasma values (Figure 4A, B).

Metabolically generated amino acids show the highest accumulation. Loader substrates are next in terms of accumulation in cells followed by harmonizer substrates, confirming their indirect mode of transport. Even in the presence of controllers, amino acids accumulate inside the cell, but in a controlled manner. This can be explained by the transport mechanism of controllers. SNAT3 and SNAT5, for example, use a mechanism that combines Na+–amino acid symporter with proton antiport (84, 85). This removes the electrical component of the Na+–electrochemical gradient, reducing accumulation to a combination of the Na+ and H+ concentration gradients, resulting in an ∼15-fold accumulation (86) beyond which net transport via SNAT3 is reversed. Cationic amino acids show the lowest accumulation (<10-fold), owing to opposing vectorial transport by the loader CAT1 and harmonizer y+LAT2. The mechanism of y+LAT2 is still incompletely understood. The charge of cationic amino acids is neutralized by cotransport of neutral amino acids with Na+ but other cations can be used as well (50). The exchange process, driven by abundant intracellular amino acids such as glutamine and alanine, would result in accumulation of cationic amino acids, but the ion dependence generates an asymmetry. Overall, y+LAT2 displays faster efflux rates of cationic amino acids than neutral amino acids (87).

**Breakdown**

Oxidation of amino acids is the main mechanism by which an excess of amino acids beyond essential replacement is removed from the blood circulation (60, 88). As outlined already, an increase of plasma amino acid concentrations translates into a corresponding increase of cytosolic amino acid concentrations. This in turn activates amino acid metabolism. Three examples of tight regulation of essential amino acid metabolism are presented here. The branched-chain keto acid (BCKA) dehydrogenase (BCKDH) is the key regulated step of BCAA catabolism, because transamination generates a rapid equilibrium between BCAA and BCKA (89, 90). The BCKDH is analogous to the pyruvate dehydrogenase complex (91). BCKDH is inhibited by phosphorylation via BCKDH kinase (BDK) and activated by dephosphorylation via a BCKDH phosphatase (PPM1K [protein phosphatase Mg2+/Mn2+ dependent 1K] aka PP2Cm [PP2C type mitochondrial protein phosphatase]). BCKAs inhibit BDK, thus activating the BCKDH and increasing BCAA oxidation (92). BDK is also transcriptionally regulated by a carbohydrate response element in its promoter (93). The BCKDH complex is inhibited by NAD(H) and branched-chain-acyl-CoA (90). BCAAs bypass the liver, owing to lack of mitochondrial branched-chain aminotransferase (BCATm) in this tissue. This allows leucine to be a more powerful activator of mTORC1 (mechanistic target of rapamycin complex 1) in peripheral tissues after a meal (94). Global knockout of BCATm causes a dramatic rise (14- to 40-fold) of BCAA concentrations in plasma (95). In addition, there is long-term regulation in response to dietary protein. When rats were fed an 8% protein diet, the isolated enzyme retained just 6% of its normal activity (96). Low-protein diets have only a small effect on plasma amino acid concentration in the fasting state (28), but this can only be maintained when amino acid metabolism is minimal and when protein intake is limiting. Elevated concentrations of BCAAs as observed in type 2 diabetes are most likely caused by reduced metabolism (5, 97). In addition to the autonomous regulation of BCAA metabolism, it is also controlled by insulin-mediated signaling in the hypothalamus, which induces BCKDH expression in liver via the autonomous nervous system (98).

The second example is phenylalanine hydroxylase (PAH), which catalyzes the first step of phenylalanine breakdown and is a tetrameric enzyme with strongly allosteric behavior. It responds in a sigmoidal fashion to phenylalanine and is regulated by phosphorylation/dephosphorylation (99, 100). More importantly, PAH is maintained in a largely inactive state by its cofactor tetrahydrobiopterin (101). This inhibition can be overcome by elevated concentrations of phenylalanine.

Tryptophan catabolism, the third example, occurs to ∼90% via the kynurenine pathway in the liver (102). The key enzyme tryptophan 2,3-dioxygenase is highly regulated and has a short half-life (∼2 h). Elevated concentrations of tryptophan...
knockout of B0AT1 (Figure 2) can normalize phenylalanine absorption in the intestine and reabsorption in the kidney by urine including small amounts of phenylalanine (106). Reducing phenylacetylglutamine, or phenylacetate. These metabolites are acid, some of which is further metabolized to phenyllactate, rise to of phenylalanine metabolism (105). It generates phenylpyruvic acid metabolite transporters. The nutritional intake of pro-

TABLE 1 Mitochondrial transporters for amino acids and their metabolites

| Gene            | Substrates | References | Mechanism | Comment                                      |
|-----------------|------------|------------|-----------|----------------------------------------------|
| SFXN1           | Ser, Gly, Ala | (153)     | n.d.      | Splice variant of SLC1A5. Glutamine metabolism not altered by SLC1A5 knock-out in other studies (65). |
| SLC1A5var       | Gin, Ala   | (154)     | A?        |                                              |
| SLC25A12 (AGC1) | Asp/Glu    | (155)     | A (Asp−/Glu− +H+) |                                              |
| SLC25A13 (AGC2) | Asp/Glu    | (155)     | A (Asp−/Glu− +H+) |                                              |
| SLC25A18 (GC2)  | Glu        | (156)     | S (Glu− +H+) |                                              |
| SLC25A22 (GC1)  | Glu        | (156)     | S (Glu− +H+) |                                              |
| SLC25A2 (ORC2)  | Orn, Cys, Lys, Arg, His | (157) | U (also A) | Oxidative metabolism |
| SLC25A15 (ORC1) | Orn, Cys, Lys, Arg | (157) | U (also A) | Urea cycle |
| SLC25A9 (ORN3)  | Orn, Lys, Arg, His | (158) | U (also A) | Oxidative metabolism |
| SLC25A38        | Gln        | (159)     | n.d.      |                                              |
| SLC25A44        | BCAAs      | (160)     | n.d.      |                                              |
| SLC25A21        | 2-oxoadipate | (161)     | A         | Tryptophan metabolism |
| MRC1/2          | 2-oxobutyrate | (162, 163) | S (H+)   | Methionine, threonine metabolism |

1 A, antipoint; AGC, aspartate/glutamate carrier; BCAA, branched-chain amino acid; Cit, citrulline; GC, glutamate carrier; MPC mitochondrial pyruvate carrier; n.d. not determined; Om, ornithine; ORC ornithine carrier; ORNT, ornithine transporter; S, symport; SFXN1, sideroflexin 1; U, uniport.

activate and stabilize the enzyme (102). This is mediated by an allosteric tryptophan binding site, occupancy of which reduces ubiquitination (103).

The critical role of catabolism is illustrated by inborn errors of amino acid metabolism such as phenylketonuria (PKU) (104) and tryptophan-2,3-dioxynogenase deficiency (102). PKU is caused by mutations of phenylalanine hydroxylase (PAH). Ingested phenylalanine is absorbed in the intestine and the excess cannot be broken down. Because amino acids are recycled efficiently, phenylalanine accumulates over time. Reference values for plasma are 35–85 μM in adults but can rise to >1000 μM in uncontrolled PKU. The upper limit is generated by phenylalanine transaminase, a minor pathway of phenylalanine metabolism (105). It generates phenylpyruvic acid, some of which is further metabolized to phenyllactate, phenylacetylglutamine, or phenylacetate. These metabolites are incompletely reabsorbed in the kidney and spill over into the urine including small amounts of phenylalanine (106). Reducing absorption in the intestine and reabsorption in the kidney by knockout of B0AT1 (Figure 2) can normalize phenylalanine concentrations in blood (106). This is caused by reduced uptake and almost complete spillover of phenylalanine into the urine. The study shows how two elements of amino acid homeostasis can be balanced against each other to ameliorate the clinical effects of PKU.

The final metabolism of amino acids into carbon dioxide and water takes place inside mitochondria, but not all require a dedicated transporter. Histidine for instance is converted to glutamate in the cytosol and aromatic amino acids are first converted to oxoacids or fumarate before oxidation inside mitochondria. Moreover, glucogenic amino acids may first be converted to glucose before complete oxidation. Table 1 lists the currently known mitochondrial amino acid/amino acid metabolite transporters. The nutritional intake of protein typically exceeds essential requirements by ∼30–70 g/d (107). As a result, an equivalent amount of amino acids is degraded.

As outlined already, catabolic pathways for essential amino acids are tightly regulated, increasing in activity as intracellular amino acid concentrations rise in response to elevated plasma concentrations. This generates transamination products (serine, cysteine, methionine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, proline, glutamate) or free ammonia (glycine, glutamine, glutamate, histidine, asparagine, threonine). If the degradation takes place in extrahepatic tissues, pyruvate is the dominant acceptor for transamination generating alanine, whereas glutamate is the acceptor for ammonia, generating glutamine. Oxaloacetate can also act as an acceptor, but the resulting aspartate remains within the cell because it is not an efflux substrate of amino acid transporters. In muscle, aspartate can be used to replenish tricarboxylic acid (TCA) cycle intermediates via the purine-nucleotide cycle (108). Alanine and glutamine are readily released across the plasma membrane because their transport processes are in equilibrium (Figure 3). Thus, an increase of intracellular alanine and glutamine concentrations, due to metabolism of other amino acids, will translate into a net efflux. This mechanism is particularly relevant in muscle, which releases alanine and glutamine during fasting (109). In the liver, glutamine and alanine are used to generate NH4+ and aspartate, respectively, which in turn are used to generate urea (110). The carbon skeleton of both amino acids is used to generate glucose. This is an important metabolic fate of amino acids during fasting, providing glucose and ketone bodies. Carbamoyl-phosphate synthetase (CPSI) is the key enzyme that regulates the speed of the urea cycle. Several mechanisms contribute to an increase of urea cycle activity in response to an amino acid load. Alanine aminotransferase generates glutamate in hepatocytes. As a result, glutamate concentrations rise rapidly upon ingestion of amino acids, although hepatocytes are not permeable to glutamate. Glutamate in turn is converted into N-acetyl-glutamate, a potent allosteric activator of CPSI. In fact, there is an almost linear relation between CPSI activity and N-acetyl-glutamate concentrations inside mitochondria (110).

### Synthesis of Nonessential Amino Acids

Nonessential amino acids can be synthesized from intermediary metabolites. As outlined already, many cells do not require transporters for aspartate and glutamate, owing to metabolic synthesis either from glutamine or from TCA cycle intermediates. Glutamine and asparagine can also be synthesized from...
glutamate and aspartate, respectively. The source to generate the carboxylamide is NH$_4^+$ in the case of glutamine and glutamine in the case of asparagine. Serine is generated from intermediates of glycolysis by a 3-enzyme pathway. The transcription of all 3 enzymes is upregulated upon serine or glutamine starvation (111). This response is mediated by the GCN2–ATF4 (General control nonrepressible 2/Activating transcription factor 4) pathway, which responds to nutrient limitation (see below). Asparagine synthetase (ASNS) is a well-known example of gene regulation by amino acid response elements (112). Similarly to glutamine, asparagine can be used as an exchange substrate to bring in additional amino acids through harmonizers (113), presumably because its role as an anaplerotic substrate for the TCA cycle is limited. Silencing of ASNS caused reduced uptake of serine and cationic amino acids, suggesting a role as an exchange substrate for the mTORC1 complex (TSC1/2), which prevents its inhibitory action on ULKs-ATG13-FIP200 form a stable complex, the activation of which is essential for autophagy (125). Autophagosome formation is induced during nutrient deprivation. Inactivation of mTORC1 by rapamycin stimulates autophagy. Induction of autophagy is slow and replenishment of amino acids through this mechanism requires hours (127).

Autophagy is, however, essential for protein biosynthesis in early embryonic stages (128). The ubiquitin-proteasomal system for protein recycling can provide amino acids on a shorter time scale (129). The system is constitutively active owing to protein turnover and misfolding. It has been estimated that 30% of newly formed proteins are immediately degraded (130). However, inhibition of the proteasome reduced translation only when ≥1 essential amino acid was reduced to 1 μM in the medium (129). The effect was reduced when amino acid starvation was prolonged, allowing autophagy to set in. In NIH3T3 cells, inhibition of the proteasome resulted in reduction of asparagine/aspartate and cysteine by 20%–30%. Supplementation with cysteine increased cell survival and markedly reduced the ISR. Consistently, cell survival was reduced in media lacking cysteine and asparagine when proteasome function was inhibited (131).

### Sensing Amino Acids

Sensing of amino acids occurs directly and indirectly. The most important principles are allosteric regulation of enzymes, amino acid binding proteins, transceptors, and tRNA binding. For short-term control of amino acid concentrations, allosteric regulation of metabolizing enzymes is probably the most important mode of regulation (88). In the absence of amino acid intake, amino acid metabolism is strictly limited. Allosteric control can be exerted by amino acids or their metabolites as outlined earlier.

Amino acid binding proteins work in conjunction with mTORC1 to sense cytosolic concentrations of arginine and leucine (132, 133). Sestrin 2 has been identified as a leucine sensor. When it binds leucine, sestrin 2 dissociates from GAP (GTPase activating protein) activity towards the Rags (134). It binds to GATOR (Guo an activator of Rag3) 2, which in turn phosphorylates the rag mammalian target of rapamycin (mTOR) complex (135). The GATOR2 complex then recruits the mTORC1 complex to the surface of lysosomes, where it can be activated by Rheb (Ras homolog enriched in brain), which is also anchored in the lysosomal membrane. To recruit mTORC1 to the membrane the nucleotide state of Rag proteins must change, which is regulated by GATOR proteins. SAR1B (secretion associated ras-related GTPase 1B) has been identified as an additional leucine sensor (134). It binds to GATOR2 under conditions of amino acid deficiency. Moreover, leucyl-tRNA synthetase has been shown to act as another leucine sensor (135).

The cytosolic arginine sensor CASTOR1 also inhibits mTORC1 through its interaction with GATOR2. As in the case of sestrin-2, binding of arginine causes dissociation of CASTOR1 from GATOR2. Another amino acid sensor is
| Gene        | Substrates               | References | Mechanism                      | Comment                                      |
|-------------|--------------------------|------------|--------------------------------|----------------------------------------------|
| SLC36A1     | Pro, Gly, Ala            | (164)      | $S\ H^+$                       |                                              |
| SLC38A7     | Gin, Asn                 | (165)      | $S\ H^+$ or $U$                |                                              |
| SLC38A9     | Phe, Leu, Ile, Thr, Met, Tyr, Val, Pro | (126, 166) | $U$                            | Transceptor for Arg                          |
| SLC7A5      | Large neutral AAs        | (167)      | $A$                            | DRAM1-dependent relocalization or LAPTM4b-dependent relocalization |
| SLC7A14     | Arg, Lys, Orn            | (169)      | $U$                            |                                              |
| SLC1A5      | Small neutral AAs        | (167)      | $A$                            | DRAM1-dependent relocalization               |
| SLC15A4     | His, peptides            | (170)      | $S\ H^+$                       |                                              |
| SLC38A1/PQLC2 | Arg, Orn, His, Lys    | (171, 172) | $U$                            |                                              |
| Cystinosin  | Cystine                  | (173)      | $S\ H^+$                       |                                              |

1A, antiport; AA, amino acid; DRAM1, DNA damage regulated autophagy modulator 1; LAPTM4b, lysosome-associated transmembrane protein 4 beta; Orn, ornithine; PQLC2, PQ-loop repeat containing protein 2; S, symport; U, uniport.

SAMTOR (S-adenosylmethionine sensor upstream of TORC), which binds S-adenosylmethionine (SAM) and relieves its inhibitory action on mTORC1 (136). SAM is an important methyl-group donor and the first step in the oxidation of methionine. The mTORC1 complex primarily regulates protein translation and autophagy, but through ATF4 also increases transcription of genes involved in amino acid transport (137).

The term “transceptor” refers to the dual nature of certain membrane proteins both acting as a transporter and being capable of initiating signal transduction like a receptor (138, 139). Although transporters undergo conformational changes suitable to signaling, the actual mechanism remained elusive until recently. In the case of SLC38A9 (SNAT9), the N-terminus of the protein is disordered and can form a loop that inserts itself into the transporter, similar to the ball and chain model of ion channel inactivation (140, 141) (Figure 5). Upon binding of amino acids, presumably to the opposite side of the transporter, conformational changes release the N-terminus, which can then bind to the gap at the interface of the RagA/RagC heterodimer. This affects the nucleotide status of the heterodimer, allowing mTORC1 to bind and become activated. The amino acid that is optimal for sensing does not necessarily coincide with the amino acids that are transported optimally (126).

Imbalances of intracellular amino acid concentrations are sensed via uncharged tRNA molecules (142). Uncharged tRNA molecules are generated after the peptidyl-transferase reaction at the ribosome. Typically, they are immediately regenerated through a variety of amino acyl tRNA synthetases, but the ratio between uncharged and aminoacylated tRNAs changes as a result of amino acid starvation (143, 144). Accumulation of uncharged tRNAs is detected by the protein kinase GCN2. It was first observed in yeast that GCN2 has a domain homologous to histidyl-tRNA synthetases (145). This domain was subsequently shown to bind uncharged tRNAs, thus presenting a general mechanism for the detection of amino acid limitation without directly monitoring individual amino acid concentrations (146). However, the affinity of tRNA synthetases for their cognate amino acid is very high, rendering a bulk increase of uncharged tRNAs difficult to achieve. Thus, advanced models of GCN2 activation invoke localized tRNA concentrations and activation by ribosome stalling (147). Activated GCN2 phosphorylates eIF2α, thereby reducing CAP-dependent translation initiation (148). At the same time, certain messenger RNAs containing upstream open reading frames (uORFs) are induced, such as the transcription factor ATF4 (149) and the amino acid transporter SNAT2 (150). The presence of uORFs results in a sequence of translation initiation–termination–reinitiation processes, which depend on the presence of eIF2-GTP. Phosphorylated eIF2(αP) acts as a competitive inhibitor of eIF2B, which prevents the recycling of eIF2-GDP into eIF2-GTP. This reduces overall translation, but at the same time favors reinitiation on open reading frames.

![FIGURE 5](https://academic.oup.com/jn/advance-article/doi/10.1093/jn/nxab342/6412706) How transceptors regulate mTORC1. The N-terminus of SLC38A9 is normally embedded in the protein. It can be released by binding/transport of AAs. The N-terminus is liberated to bind to the RagA/RagC heterodimer, inducing a conformation in which the nucleotide status changes. This causes mTORC1 to bind to the lysosomal surface. Image generated with Biorender. AA, amino acid. mTORC1, mechanistic target of rapamycin complex 1.
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Conclusion

The elements of amino acid homeostasis act together during the feeding–fasting cycle. Upon nutrient intake, amino acids are absorbed in the intestine, which results in elevated concentrations of amino acids in the plasma and, via transport processes, this raises amino acids proportionally in the cytosol. In muscle, protein synthesis is activated through mTORC1 and insulin. Rising amino acid concentrations activate amino acid metabolism in all tissues. This will generate glutamine (from ammonia) and alanine (from transamination), which are absorbed in the intestine, which results in elevated concentrations. This is effectively demonstrated in inherited gluconeogenesis.

Metabolism is the primary mode by which an excess of amino acids can be translated into elevated cytosolic amino acid concentrations. This is effectively demonstrated in inherited disorders of amino acid metabolism. In these extreme cases, the efficient recycling and reabsorption of amino acids acts as a trap causing excessive accumulation of these solutes over time. Only at very high concentrations do the affected amino acids spill over into the urine or they are degraded by noncanonical pathways. Dysregulated amino acid concentrations also have the potential to serve as biomarkers for other diseases, such as diabetes where the elevation of BCAAs is an early sign of developing insulin resistance and reduced metabolism.

A detailed understanding of amino acid homeostasis can improve human health in a variety of disease states. Metionine restriction is being considered as an enhancement of cancer therapy. Inhibition of tryptophan-degrading enzymes or tryptophan restriction is being considered as an enhancement of cancer treatment. Inhibition of tryptophan-degrading enzymes or tryptophan restriction is being considered as an enhancement of cancer treatment. Inhibition of tryptophan-degrading enzymes or tryptophan restriction is being considered as an enhancement of cancer treatment.

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