REVIEW ARTICLE

Amino acid transporters: roles in amino acid sensing and signalling in animal cells

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Amino acid availability regulates cellular physiology by modulating gene expression and signal transduction pathways. However, although the signalling intermediates between nutrient availability and altered gene expression have become increasingly well documented, how eukaryotic cells sense the presence of either a nutritionally rich or deprived medium is still uncertain. From recent studies it appears that the intracellular amino acid pool size is particularly important in regulating translational effectors, thus, regulated transport of amino acids across the plasma membrane represents a means by which the cellular response to amino acids could be controlled. Furthermore, evidence from studies with transportable amino acid analogues has demonstrated that flux through amino acid transporters may act as an initiator of nutritional signalling. This evidence, coupled with the substrate selectivity and sensitivity to nutrient availability classically associated with amino acid transporters, plus the recent discovery of transporter-associated signalling proteins, demonstrates a potential role for nutrient transporters as initiators of cellular nutrient signalling. Here, we review the evidence supporting the idea that distinct amino acid "receptors" function to detect and transmit certain nutrient stimuli in higher eukaryotes. In particular, we focus on the role that amino acid transporters may play in the sensing of amino acid levels, both directly as initiators of nutrient signalling and indirectly as regulators of external amino acid access to intracellular receptor/signalling mechanisms.

Key words: general control 2 (GCN2), integrin, mammalian target of rapamycin (mTOR), System A transporter 2 (SAT2).

INTRODUCTION

Certain nutrients have the capability to regulate cell function beyond their essential role in metabolism. In lower eukaryotes and prokaryotes, complex control pathways have been delineated by which the availability of a nutrient may regulate the acquisition, synthesis and utilization of itself (and also other nutrients) as well as modulate general metabolic processes within the cell [1]. In contrast, for higher eukaryotes, it has conventionally been thought that endocrine and neuronal systems play a dominant role governing the response of tissues to altered nutrient availability, at least *in vivo*. However, there is now mounting evidence (largely from studies in mammalian cells) that certain nutrients, possibly acting through specific nutrient receptor or 'sensor' mechanisms, have the capability to initiate cell-signalling events and regulate gene expression in the absence of hormonal influences. These nutrients, which include amino acids [2], glucose [3], fatty acids [4], sterols [5] and iron [6], may also themselves contribute substantially to the regulation of certain endocrine mechanisms [7]. For example, glucose and amino acids are potent modulators of both insulin and glucagon secretion and may also participate in the release of more recently identified hormones such as the glucagon-like peptides [8–11].

The regulation of cell function by amino acids in higher eukaryotes has become the focus of considerable interest, and it is now well-recognized that some members of this heterogeneous group of organic molecules exert powerful regulatory control over fundamental cellular processes such as the synthesis and degradation of protein, glycogen and lipid [12]. Numerous studies have demonstrated that elevated amino acid availability generally sustains anabolism and inhibits catabolism in eukaryotic cells (reviewed in [13–17]). Thus global protein synthesis is increased and proteolysis is at least relatively decreased when the precursors for protein synthesis are abundant. Increased amino acid availability can also increase global mRNA abundance, by enhancing both transcriptional rates and mRNA stability [13,18,19]. When amino acids are scarce, the above effects are reversed, although the synthesis, stability and translation of select gene transcripts (notably those involved in the biosynthesis or transport of amino acids) may be increased upon amino acid deprivation [13,20] by specific mechanisms which oppose the global changes in gene expression [21].

Our knowledge of the downstream signalling and gene-expression mechanisms modulated in response to altered amino acid availability [e.g. the highly-conserved eukaryotic target of rapamycin (TOR) and general control 2 (GCN2) pathways] is becoming better defined in the wake of recent discoveries (as summarized in Figure 1). In sharp contrast, our understanding of the nature of the cellular machinery involved in amino acid sensing and signal initiation is much less secure [14]. For example,

Abbreviations used: eIF, eukaryotic initiation factor; 4E-BP, eIF4E-binding protein; AIB, α-aminoisobutyric acid; ALS, amyotrophic lateral sclerosis; AMPK, AMP-activated protein kinase; APC, amino acid–polyamine–choline; CaR, calcium receptor; CAT, cationic amino acid transporter; CHO, Chinese-hamster ovary; DAT, dopamine transporter; EAAT, excitatory amino acid transporter; GABA, γ-aminobutyric acid; GAT, GABA transporter; GCN, general control; GLYT, glycine transporter; GTRAP, glutamate-transporter-associated protein; Hic-5, hydrogen peroxide-inducible clone-5; HSP, heat-shock protein; JNK, c-Jun N-terminal kinase; LAT, System L amino acid transporter; Leu8-MAP, Leu_8-Lys_4-Lys_2-Lys-βAla; LIM, Lin-11, Isl-1 and Mec-3; MAP, microtubule-associated protein; MAPK, mitogen-activated protein kinase; Me-AIB, α-methylaminoisobutyric acid; (e)NOS, (endothelial) nitric oxide synthase; PFK1, 6-phosphofructo-1-kinase; PFK2, 6-phosphofructo-2-kinase; SH3, Src homology 3; S6K, ribosomal protein S6 kinase; SAT, System A transporter; SLC, solute carrier; (m)TOR, (mammalian) target of rapamycin; VGLUT, vesicular glutamate transporter.

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although signalling by the protein kinase mammalian (m) TOR is exquisitely sensitive to amino acid availability, there is no evidence that the kinase is regulated directly by amino acids [22], implying that the initial sensing and signalling events lie upstream of mTOR itself – thus mTOR serves as a component of the effector pathway, modulating cell functions in response to altered nutrient levels. Here we review the evidence supporting the idea that distinct amino acid 'receptors' function to detect and transmit certain nutrient stimuli in higher eukaryotes. We will focus particularly on the role that amino acid transporters may play in the sensing of amino acid levels, both directly as initiators of nutrient signalling and indirectly as regulators of external amino acid access to intracellular receptor mechanisms.

Elucidation of the mechanisms by which cells of higher eukaryotes sense changes in amino acid availability may have important therapeutic applications, since there are many pathological circumstances associated with dysregulation of amino acid metabolism for which nutritional or pharmacological intervention through such mechanisms may be of clinical benefit. Examples include (i) protein wasting observed in the musculature upon limb immobilization, disease, stress and injury which develops in parallel with depletion of free intramuscular glutamine [23], (ii) toxic over-accumulation of synaptic glutamate observed in amyotrophic lateral sclerosis (ALS) [24,25] and (iii) increased proliferation and invasiveness of tumour cells resulting from changes in amino acid availability and transport [26]. In addition, amino acid abundance may regulate glucose disposal and the cellular response to insulin, implicating amino acid-sensing mechanisms as a possible contributing factor to the development of insulin resistance and potentially to type II diabetes mellitus. For example, amino acids stimulate glycogen synthesis in hepatocytes and skeletal muscle [27–29], leucine stimulates glucose transport in skeletal muscle [30] and amino acids co-operate with insulin to both stimulate protein synthesis and inhibit protein degradation in many tissues [12,31–37]. In contrast, several recent studies have demonstrated that elevated amino acid availability may exert a negative influence upon insulin-stimulated muscle and adipose-tissue glucose uptake [38–42] and may desensitize hepatic gluconeogenesis to insulin [43]. Amino acids may exert these negative effects through the inhibition of several early steps in the insulin signalling cascade [31,40,41].

MAMMALIAN AMINO ACID TRANSPORTERS

Mammalian cells have a broad range of mechanisms for the transmembrane transport of amino acids [44–50]. Amino acid transporters have been classified into distinct 'systems' dependent upon substrate specificity, transport mechanism and regulatory properties. The proteins responsible for many of these transport activities have now been identified by molecular cloning, as summarized in several extensive recent reviews [44–46,48–50]. Table 1 summarizes some of the mammalian plasma-membrane amino acid-transport systems that have been described and their respective transporter genes (where known). We will briefly review basic features of a number of these transport systems, as their characteristics are relevant to our discussion of nutrient sensing and signalling.

Intracellular amino acid concentrations are generally higher than (or at least equal to) those in extracellular fluid, and active transport mechanisms are used to concentrate many amino acids in the cell. Several amino acid transporters (e.g. Systems A, N and X^-_{AG}) couple amino acid flux with thermodynamically competent Na^+ influx to catalyse uphill amino acid movement by secondary active transport [44–47]. The activity of the Na^+/K^+-ATPase helps maintain the transmembrane gradient for Na^+ far from equilibrium. Some transport activities are also coupled to gradients of other ions such as K^+, H^+ and OH^- [45]. Transmembrane potential difference is used as a driving force for cationic amino acid influx through System y^+. Figure 2 shows how the activities of amino acid transporters discussed in this Review are coupled to electrochemical gradients, facilitating amino acid acquisition. A net movement of electrical charge occurs as certain amino acid transporters progress through their transport cycle, which may directly affect the membrane potential.

The uptake of a particular amino acid into a specific cell type may occur via several different transport systems. For example, glutamine can be transported into hepatocytes by at least two secondary active transporters, namely System A and System N, and by the System ASC exchanger [49]. Amino acid exchangers such as Systems L, ASC and y^+L may serve as tertiary active transporters, utilizing transmembrane amino acid concentration gradients generated by secondary active transport to facilitate the transport of other amino acids against a concentration gradient [45,51]. Exchange-mediated transport through some transporters (System ASC and y^+L) may also be dependent on transmembrane electrical gradients. System L operates as an obligatory 1:1 amino acid exchanger which can couple the cellular uptake of essential branched-chain and aromatic amino acids with the efflux (by hetero-exchange) of cytoplasmic amino acids such as glutamine [51]; thus net cellular accumulation (or depletion) of specific System L substrates is possible, without any overall change in the total amino acid concentration on either side of the cell membrane. The efficient operation of this type of tertiary active transporter is likely to be heavily dependent upon the activity (in parallel) of secondary active transporters such as System A and System N, in order to provide continued supply of short-chain neutral amino acids (including glutamine) for hetero-exchange. This proposition is supported by the finding that prolonged exposure of cells to α-methylaminoisobutyric acid (Me-AIB), a synthetic System A substrate which competes for accumulation within the cell but does not efflux through tertiary active transporters, reduces cellular levels of System L substrates [52].

AMINO ACID TRANSPORTERS AND GENERATION OF NUTRIENT SIGNALS

Cells have developed discrete chemosensory mechanisms for a variety of nutrients. These mechanisms may involve either the binding of nutrients (or their metabolites) to plasma-membrane or intracellular receptors, or the detection of physiological signals generated as a result of the transmembrane transport of the nutrient (e.g. changes in cell volume or cell membrane potential). These nutrient sensors act to regulate signal-transduction cascades, and therefore nutrient 'sensing' may culminate in the altered expression and/or activity of a multitude of cellular proteins. A complicating factor concerning studies of cellular nutrient sensors is the potential occurrence of several distinct receptors for an individual nutrient within the same cell (for an example, see [53]).

Figure 3 summarizes the ways by which amino acid transporters might contribute to initiation of amino acid-dependent cell signalling. These fall into two major categories: (1) the transporter may initiate cellular signalling in direct response to (or as a direct consequence of) changes in substrate loading or flux (Figures 3A and 3B) or alternatively (2) it may regulate the availability of an amino acid to a specific pool of nutrient receptors (Figures 3C and 3D).

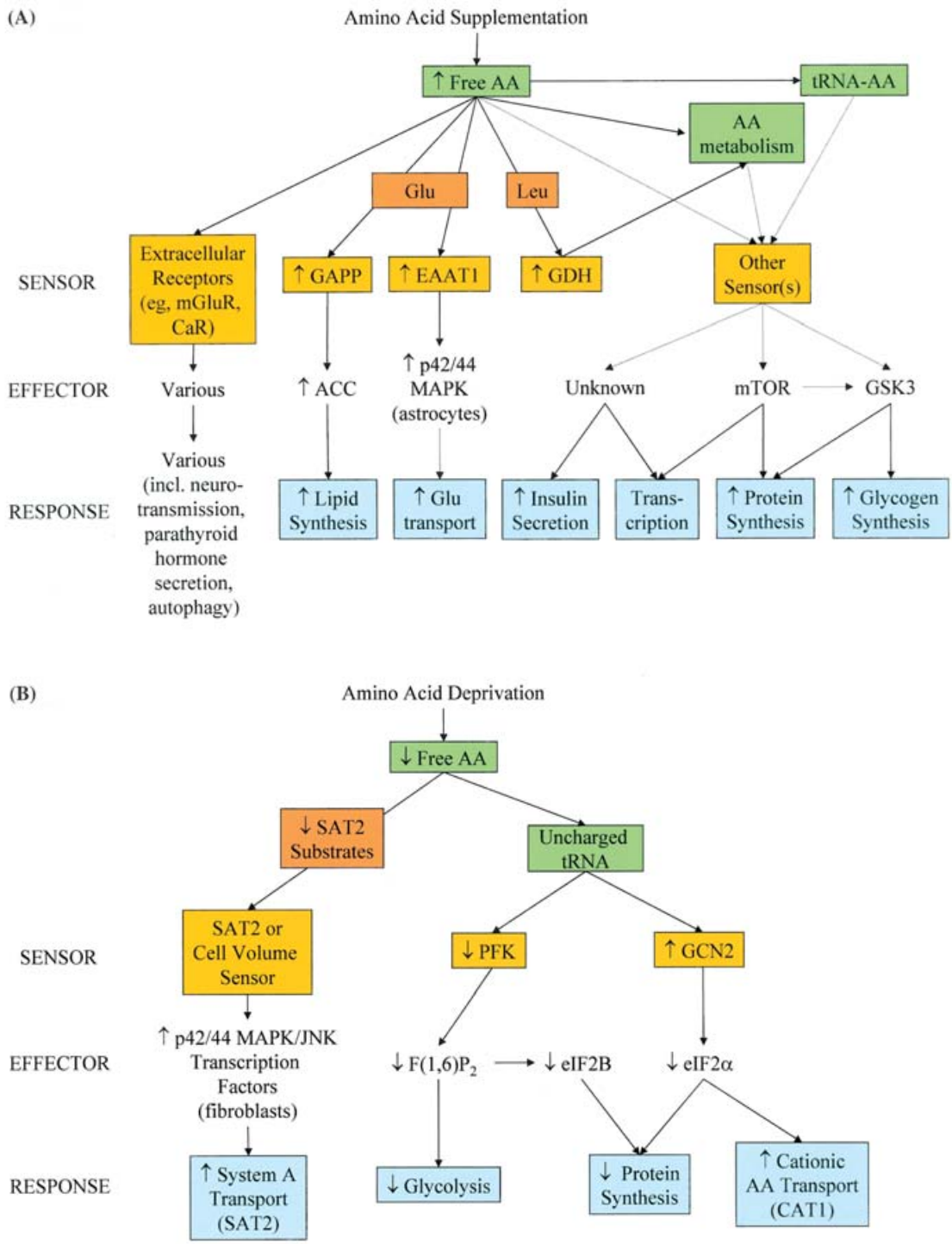

Figure 1 Mechanisms by which amino acids ('AA') are known to modulate animal cell signalling and gene expression

Studies of the response of mammalian cells to amino acid supplementation (**A**) and/or deprivation (**B**) have implicated a number of pathways in amino acid signalling (discussed in the text). Several reviews covering aspects of these pathways have recently been published and the reader is encouraged to look at these [2,8,14,46,53,92,181,187,209–215]. Amino acids and/or the cellular products of their metabolic conversion (eg, ATP, AA-tRNA; green boxes) are sensed by cellular proteins ('SENSOR'; yellow boxes) that initiate signalling via a number of pathways ('EFFECTOR') that culminate in altered cellular function ('RESPONSE'; blue boxes). Orange boxes indicate known roles for specific amino acids (or groups of amino acids). Where a direct pathway can be traced between an upstream stimulus and a downstream response, a continuous line is used [e.g. glutamate activates glutamate-activated protein phosphatase (GAPP)]. In other instances the pathway is less well characterized, and broken lines connect upstream components to downstream responses. This diagram is by no means an exhaustive review of the pathways indicated; for example, although an extracellular amino acid sensor has been implicated in the regulation of hepatic autophagy [14], intracellular amino acid levels may also play a regulatory role in liver and other tissues. Additional abbreviations: $F(1,6)P_2$, fructose-1,6-bisphosphate; mGluR, metabotropic glutamate receptor; GDH, glutamate dehydrogenase; GSK3, glycogen synthase kinase-3; ACC, acetyl-CoA carboxylase.

Table 1 Amino acid transport systems of mammalian cells

Transmembrane amino acid transport is catalysed by a number of discrete systems. The genes encoding and the proteins responsible for these transport activities are presented (where known). Amino acid transporters have been extensively reviewed elsewhere and the reader is encouraged to refer to publications cited in the text. *, Holotransporter formed upon association with the CD98 glycoprotein encoded by the gene SLC3A1; **, holotransporter formed upon association with the rBAT glycoprotein encoded by the gene SLC3A2. BCH, α-aminoendobicyclo[2,2,1]heptane-2-carboxylic acid.

(ai) Neutral-amino-acid transporters: sodium-dependent

System	Protein	Gene	Amino acid substrates (one-letter code)	Notes
A	SAT1 SAT2 SAT3	SLC38A1 SLC38A2 SLC38A4	G, A, S, C, Q, N, H, M, T, Me-AIB, P, Y, V G, P, A, S, C, Q, N, H, M, Me-AIB G, P, A, S, C, N, M, H, K, R	Short-chained-neutral-amino-acid transport. Sensitive to low pH. Ubiquitous expression. SAT3 may also function as a Na^+-independent cationic amino acid transporter.
ASC	ASCT1 ASCT2	SLC1A4 SLC1A5	A, S, C A, S, C, T, Q	High-affinity short-chain-amino-acid exchanger. Ubiquitous expression.
B^0	ASCT2	SLC1A5	A, S, C, T, Q, F, W, Y	Broad substrate specificity. Expressed on apical surface of many epithelia. May include novel isoforms of ASCT2 (e.g. a novel gene recently characterized by Pollard et al. [216]).
BETA	GAT1 GAT2 GAT3 BGT1 TAUT	SLC6A1 SLC6A13 SLC6A11 SLC6A12 SLC6A6	GABA GABA, betaine, P, β-A GABA, betaine, taurine GABA, betaine Taurine	Widely expressed Cl^--dependent GABA, betaine and taurine transporters.
Gly	GLYT1 GLYT2	SLC6A9 SLC6A5	G, sarcosine G, sarcosine	Na^+- and Cl^--dependent high-affinity glycine transport. Expressed in brain and some non-neural tissues.
IMINO	–	–	P	Na^+-dependent eptihelial proline transporter, inhibited by Me-AIB.
N	SN1 SN2	SLC38A3 SLC38A5	Q, N, H Q, N, H, S, G	Li^+-tolerant transport of Gln, Asn and His. H^+ antiport. Li^+-intolerant variants described
N^m	–	–	Q, N, H	
N^b	–	–	Q, N, H	
PHE	–	–	F, M	Brush-border transporter for Phe and Met
PROT	PROT	SLC6A7	P	Proline transporter in central nervous system.

(aii) Neutral-amino-acid transporters: sodium-independent

System	Protein	Gene	Amino acid substrates	Notes
asc*	Asc1 Asc2	SLC7A10	G, A, S, C, T G, A, S, T	Small neutral AA exchanger.
imino	PAT1/LYAAT1 PAT2/LYAAT2	SLC36A1 SLC36A2	P, G, A, β-A, GABA Me-AIB P, G, A, β-A, GABA Me-AIB	H^+-coupled transport of small neutral amino acids. Inhibited by Me-AIB.
L*	LAT1 LAT2	SLC7A5 SLC7A8	H, M, L, I, V, F, Y, W, Q A, S, C, T, N, Q, H, M, L, I, V, F, Y, W	Ubiquitously expressed exchanger for large hydrophobic amino acids.
T	TAT1	SLC16A10	F, Y, W	Aromatic-amino-acid transporter. H^+/monocarboxylate transporter family – insensitive to pH, however.

(bi) Anionic-amino-acid transporters: sodium-independent

System	Protein	Gene	Amino acid substrates	Notes
X^-_{AG}	EAAT1 EAAT2 EAAT3 EAAT4 EAAT5	SLC1A3 SLC1A2 SLC1A1 SLC1A6 SLC1A7	E, D E, D E, D, C E, D E, D	Widespread Glu and Asp transporter. K^+ antiport. Substrate-dependent uncoupled anion flux. Lack of stereospecificity toward Asp.

(bii) Anionic-amino-acid transporters: sodium-independent

System	Protein	Gene	Amino acid substrates	Notes
x^{-c}*	xCT	SLC7A11	E, Ci	Electroneutral Glu/cystine exchanger.
–	XAT2	–	D, E	Non-functional upon 4F2hc/rbAT heavy-chain co-expression. Predicted to associate with a novel glycoprotein.

Table 1 (Contd.)

(ci) Cationic-amino-acid transporters: sodium-dependent

System	Protein	Gene	Amino acid substrates	Notes
$B^{0,+}$	ATB(o,+)	SLC6A14	K, R, A, S, C, T, N, Q, H, M, I, L, V, F, Y, W	Blastocysts and possibly brush-border membrane. Broad specificity for neutral and cationic amino acids. Accepts BCH.
y^+L^*	y + LAT1 y + LAT2	SLC7A7 SLC7A6	K, R, Q, H, M, L K, R, Q, H, M, L, A, C	Na^+-dependent cationic/neutral-amino-acid exchanger. Electroneutral.

(cii) Cationic-amino-acid transporters: sodium-independent

System	Protein	Gene	Amino acid substrates	Notes
$b^{0,+***}$	b(o,+)AT	SLC7A9	K, R, A, S, C, T, N, Q, H, M, I, L, V, F, Y, W, Ci	Broad-specificity cationic- and neutral-amino-acid exchanger.
y^+	Cat-1 Cat-2 Cat-3 Cat-4	SLC7A1 SLC7A2 SLC7A3 SLC7A4	R, K, H R, K, H R, K Unknown	Cationic-amino-acid (and Na^+-dependent neutral-amino-acid) transport. Variable degree of *trans*-stimulation.

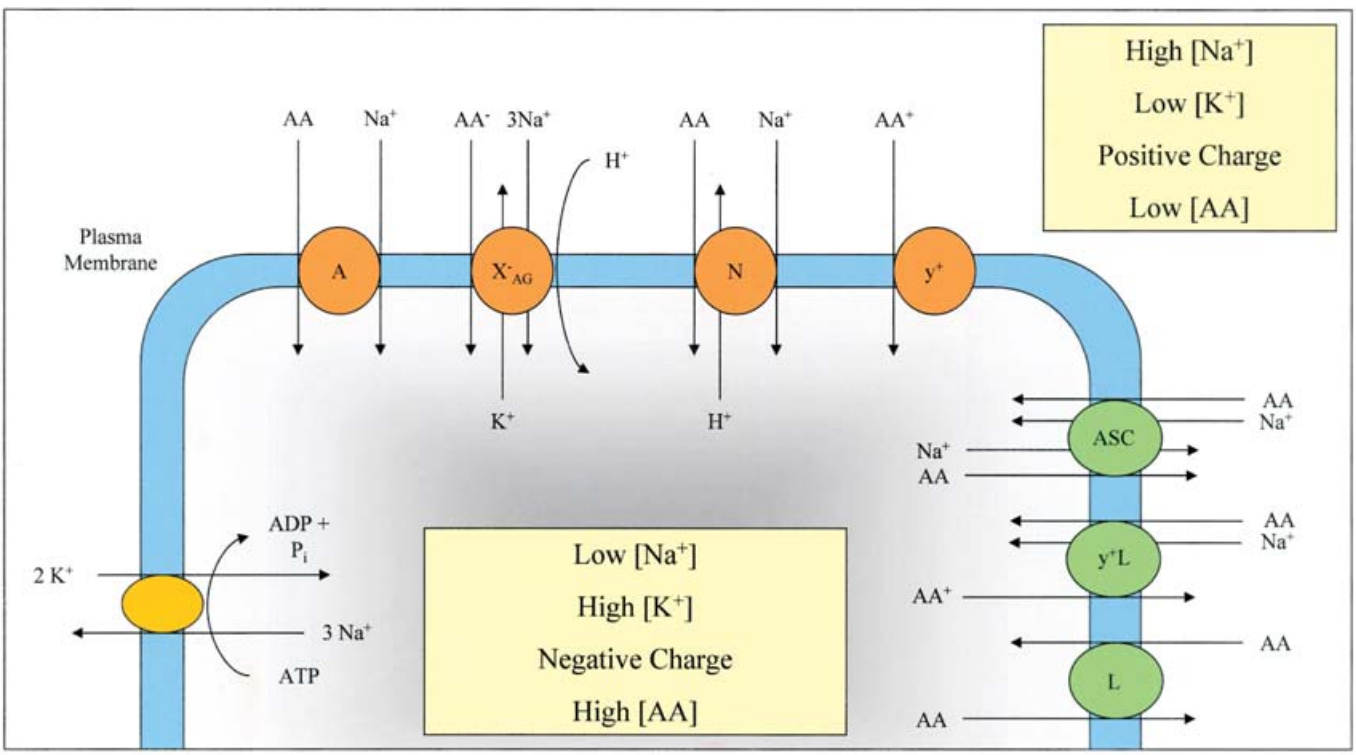

Figure 2 Active transport mechanisms involved in the maintenance of transmembrane gradients of amino acids

The amino acid transport systems presented in Table 1 differ in terms of transport mechanism, regulatory properties and substrate specificity. The Na^+/K^+-ATPase (yellow) is a primary active transporter and helps maintain transmembrane Na^+ and K^+ gradients. Secondary active transporters (browny-orange), such as Systems A, X^-_{AG}, N and y+, couple amino acid transport to the electrical and chemical gradients initiated by primary active transport. Amino acid exchangers (green), such as Systems ASC, y^+L and L, transport amino acids by antiport mechanisms. Generalized properties of the intra- and extra-cellular space are presented in yellow boxes.

In Figure 3(A), the amino acid transporter itself acts as a nutrient sensor and is responsible for initiating cellular signalling. In this scheme, the amino acid stimulus may be transduced to a chemical signal as a result of conformational transition states in the carrier protein induced as part of the transport cycle. Alternatively, simple binding of the amino acid to the transporter may in itself be sufficient to evoke a signalling event; for example, certain glutamate transporters also function as ligand-gated

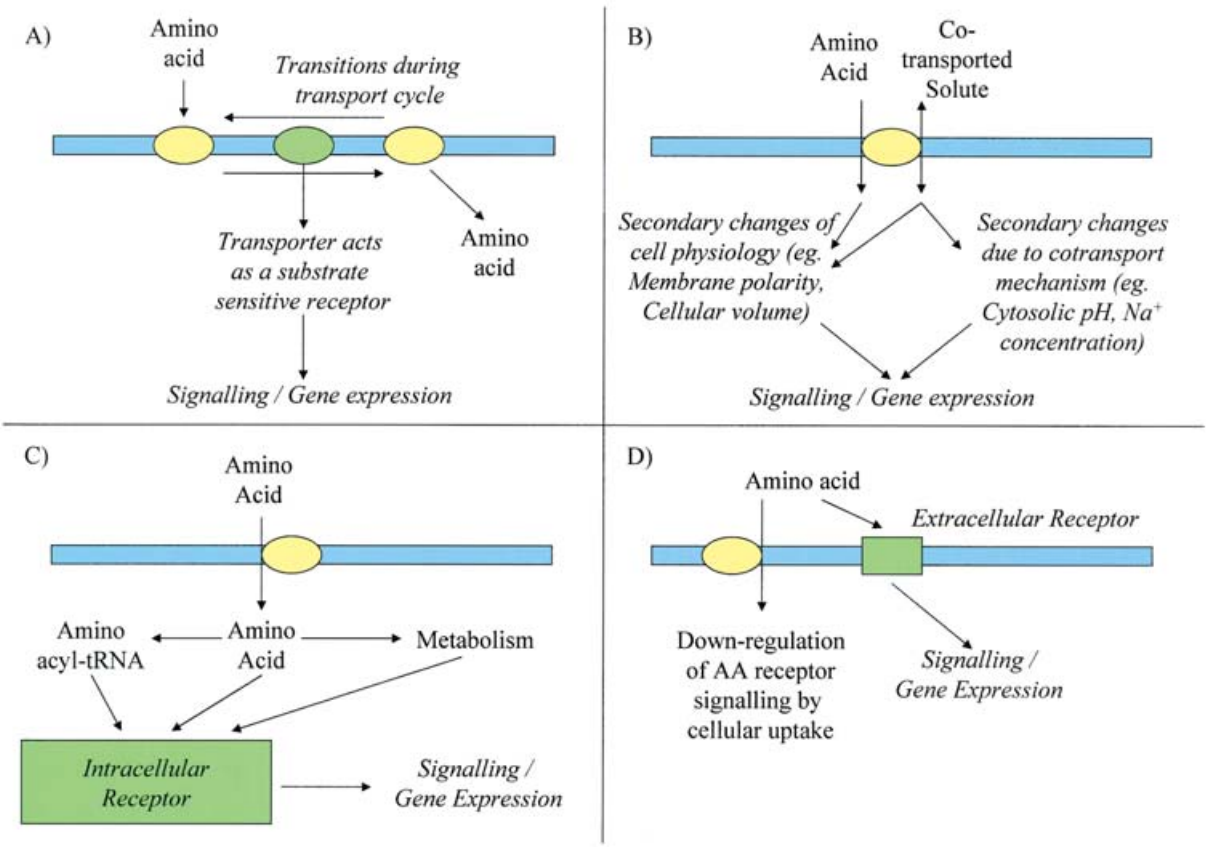

Figure 3 Potential mechanisms whereby amino acid transporters may impact on signal transduction and/or gene expression

Individual panels (**A**–**D**) are discussed in the text. In each panel, the plasma membrane is presented in blue, with the extracellular and intracellular spaces presented above and below the membrane respectively.

chloride channels [54], and this property is retained in certain non-transporting transporter mutants [55]. Most amino acid transporters function at the plasma membrane and may therefore be capable of monitoring intra- and/or extra-cellular amino acid concentrations. It is conceivable that changes in amino acid flux or availability are detected by transporters and transduced to classical signalling molecules such as the G-proteins by protein–protein interactions.

Figure 3(B) illustrates how nutrient-evoked signalling may result from secondary events associated with the specific transport mechanism of a stimulatory amino acid. As described above, the transport of amino acids against a concentration gradient is made thermodynamically possible by coupling uphill amino acid transport with downhill transport of either ions (Na^+, K^+, H^+) or other amino acids along (electro)chemical gradients. Such coupled transport may lead to passive changes within the cell (as shown in Figure 3), and transduced stimuli of these types may be ultimately responsible for initiating the signalling event. In this system, signalling is a function of the transport mechanism, and it is implicit that any transporter with an identical mechanism (e.g. 1:1 amino acid/Na^+ symport) would initiate the same response (provided equivalent transport rates are achieved). Furthermore, such responses should be mimicked by synthetic substrate analogues for the transporter, whereas non-transportable inhibitory compounds would be predicted to suppress the response of the cell to transporter substrates.

In Figure 3(C), amino acids, or the products of amino acid metabolism/modification [including ATP, amino acyl-tRNA and oxo ('keto') acids] are sensed by an intracellular receptor. Extracellular amino acid is delivered to the intracellular sensing compartment by a transporter situated at the plasma membrane. In this model, amino acid signalling correlates with the capacity of the transporter to deliver the signalling amino acid to (and perhaps concentrate it within) the cell, in relation to concurrent processes such as cellular amino acid/protein turnover and the overall sensitivity of the intracellular receptor mechanism. The actual transport mechanism and transporter isoforms used may be of lesser consequence to signalling capability, because this is determined by the intracellular concentration of a specific amino acid. Nevertheless, the overall level of transporter expression (reflecting transport V_{max}) and the relative K_m value should contribute to the responsiveness of the sensor.

In Figure 3(D), an extracellular receptor senses the availability of external amino acids, but high activity of a transporter in close proximity to the receptor influences the local amino acid concentration sufficiently to modulate initiation of signalling. Thus amino acid signalling through extracellular receptors may be attenuated by cellular amino acid uptake. This type of process is most likely to be effective in relatively discrete extracellular spaces such as the synaptic cleft, and indeed is observed with respect to the neurotransmitter glutamate in the central nervous system [25].

These four major types of mechanism are considered separately in greater detail (using specific examples) in the subsequent sections of this review.

INITIATION OF CELLULAR SIGNALLING BY AMINO ACID TRANSPORTERS: THE TRANSPORTER AS A NUTRIENT SENSOR

In higher eukaryotes, several amino acid transporters have been implicated directly in the initiation of intracellular signalling in response to altered substrate availability. Here we will focus on two representative examples, the System A (predominantly System A transporter 2; SAT2) transporter and the glial glutamate transporter EAAT1 (excitatory amino acid transporter 1). Amino acid availability regulates the activity of both of the above transporters; however, these effects occur in opposite directions – hence SAT2 expression is increased following amino acid deprivation, whereas EAAT1 is stimulated by the provision of excess amino acid [56–58]. It should be noted that several other amino acid transporters in animal cells are also regulated by amino acid availability – for example, System X^-_{AG} (probably EAAT3) activity is increased by amino acid deprivation in the kidney cell line NBL-1 [59], System L (System L amino acid transporter 1; LAT-1) is induced in hepatocytes upon limiting the cellular supply of arginine (which is not a substrate for this transporter) [60], System N^m activity is induced upon depriving rat skeletal-muscle primary cultures of glutamine [61] and the expression of the System y^+ cationic amino acid transporter (CAT1) is subject to amino-acid-sensitive regulation at a number of post-transcriptional steps [20,62–66]. In contrast with the situation with SAT2 and EAAT1, the amino-acid-sensing events involved in regulation of these transporters is poorly defined or appears to involve intracellular amino acid sensing (to be considered later in this Review).

An important feature of some amino-acid-sensitive events is the ability of non-metabolizable analogues to reproduce the effect of a natural amino acid. In such instances, metabolism of the natural amino acid appears unlikely to be a major factor in eliciting the cellular response. The synthetic compounds may act via cellular sensors present either at the plasma membrane or within the cell. However, in several examples discussed below, the cellular response to amino acids (and their synthetic analogues) is such that a direct involvement of amino acid transporters can be implied in amino acid sensing. This involvement appears to go well beyond simply regulating the access of an amino acid ligand to its intracellular sensor and is supportive of the suggestion that eukaryotic solute transporters may be capable of initiating signal-transduction pathways and regulating gene expression through mechanisms similar to many receptor–ligand interactions. An excellent example from a lower eukaryote is the yeast (*Saccharomyces cerevisiae*) protein Ssy1p, an amino acid permease homologue. Ssy1p belongs to the amino acid–polyamine–choline (APC) transporter superfamily which also includes the mammalian solute carrier 7 (SLC7) transporter family [67]. Ssy1p lies upstream of signalling mechanisms for the regulated expression of several yeast amino acid and peptide permeases and has been implicated in the sensing of extracellular amino acid availability [1]. Although Ssy1p is homologous with other yeast amino acid permeases, it is not thought to operate as an amino acid transporter, but rather as a sensor of environmental amino acid availability. This premise is based on the observation that, in a yeast strain expressing Ssy1p but lacking demonstrable D-leucine transport, D-leucine regulates β-galactosidase transcription from a Ssy1p-sensitive reporter gene [68]. However, it may not be necessary for amino acids to flux through a transporter for that protein to initiate signalling (as already suggested above in relation to Figure 3A). The binding of an amino acid substrate to (or alternatively amino acid flux through) a transporter may induce significant changes in the tertiary structure of the transporter and it is conceivable that this physical change is sensed by cellular signalling proteins that initiate a signal-transduction pathway. Such events are the basis for initiation of signalling by transmembrane receptors that lack intracellular enzymic activity (for example, the G-protein-coupled receptors) and integrins.

System A and adaptive regulation

One of the best-characterized amino-acid-sensitive processes in animal cells is the transcription-dependent increase in the activity of the neutral amino acid transporter System A in response to amino acid deprivation (a process referred to as 'adaptive regulation' or 'derepression'). There are three proteins currently identified that exhibit System A activity (SAT1–3), of which SAT2 is the most abundantly expressed. The molecular mechanisms involved in adaptive regulation were studied for many years prior to the cloning of the proteins responsible for System A activity, and many hypotheses have been proposed to explain the upstream sensing event regulating this phenomenon [69–74]. Recent work from our group and others has now demonstrated directly that the adaptive regulation of System A activity occurs concomitant with an increase in the expression and cell-surface abundance of the System A transporter (SAT2) protein [56,57,75].

It was initially proposed by Gazzola et al. [70] that the ability of amino acids to inhibit adaptive regulation may relate to their ability to bind to the System A transporter. System A was therefore the first mammalian membrane protein suggested to act as both a transporter and receptor. Me-AIB is a specific substrate for System A transporters. During amino acid deprivation, SAT2 mRNA increases and this increase is suppressed by Me-AIB (as well as by natural System A substrates, such as asparagine, glutamine and proline) [56,57]. Non-System A (SAT2) substrates, including glutamate and arginine, were shown not to inhibit the increase in SAT2 mRNA. Two proposals can be suggested for the regulation of SAT2 expression by System A substrates – either a SAT2-regulating amino acid receptor exists that has a very similar ligand specificity to SAT2, or SAT2 acts as a substrate receptor for regulation of its own expression. This latter proposition echoes the initial suggestion of Gazzola et al. [70]. To function effectively as a receptor, a nutrient-sensitive protein would be expected to undergo predictable changes in conformation, association with other proteins or catalytic activity in response to physiologically relevant increments or decrements in nutrient concentration. Thus it would be anticipated that any amino acid receptor or transporter acting as a nutrient 'sensor' would possess a ligand-binding constant or (with respect to nutrient transport) a Michaelis–Menten constant (K_m) for the nutrient equal to, or greater than, the normal physiological substrate concentration (thus affording an effectively linear increase in activation state of the sensor over the expected range of substrate concentrations). SAT2 exhibits K_m values for its neutral amino acid substrates within the concentration range observed in extracellular fluid, consistent with the idea that this System A transporter may act as a nutrient sensor upstream of the (as yet largely unidentified) signalling mechanisms regulating the adaptive regulatory response. There is no evidence that the amino-acid sensitive mTOR or GCN2 pathways (Figure 1) contribute directly to the processes underlying adaptive regulation of SAT2. The p42/44 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) pathways may have a role in adaptive regulation in certain cell types (e.g. fibroblasts [76]), although in

this case it appears that amino acid deprivation may be sensed at least partly as the passive reduction in cell volume occurring as an osmotic consequence of reduced amino acid uptake through the System A carrier (as described in the next subsection). In contrast, there is considerable evidence that amino acid deprivation and cell shrinkage (hypertonic shock) exert their effects upon System A activity through different signalling pathways in many cell types. For example, stimulation of System A by hypertonicity in the epithelial cell line NBL-1 is sensitive to microtubule disruption, whereas the adaptive response is insensitive to such treatment [77,78]. Furthermore, for mutant Chinese-hamster ovary (CHO) cells in which System A activity does not respond to amino acid deprivation, a stimulation by hypertonic stress is maintained. These latter observations highlight the importance of additional mechanisms independent of cell-volume change for adaptive regulation of System A, which may be directly responsive to signals initiating from the transporter itself. The effector mechanisms for adaptive up-regulation of System A include SAT2 transporter recruitment from intracellular pools [56] as well as increased SAT2 gene transcription [79].

Following prolonged exposure of cells to an amino acid-free environment, System A exists in a high-activity de-repressed state (reviewed in [80]). Upon re-supplementation of amino acids, the initial decline in transport activity is independent of changes in gene expression (*trans*-inhibition) and precedes a further reduction in System A activity that is dependent upon gene expression (repression). *Trans*-inhibition is an apparently unique characteristic of System A transporters and represents the ability of cytoplasmic amino acids to inhibit the uptake of extracellular amino acids through the transporter. It has been proposed that binding of either cytoplasmic amino acids or Na^+ ions (or both) to the transporter prevents the completion of the transport cycle, which requires unoccupied substrate-binding sites to return to the extracellular face of the membrane. The incorporation of *trans*-inhibition into the System A transport mechanism may allow dynamic regulation of both amino acid uptake rate and amino-acid-induced signalling by changes in System A substrate abundance on either side of the plasma membrane.

Glutamate signalling and EAAT1 in astrocytes

Another amino acid transporter implicated in the initiation of intracellular signalling in response to altered substrate availability is the glial glutamate transporter EAAT1. There is considerable evidence indicating that the interaction of glutamate with EAAT1 may regulate glial signal transduction and morphology [81] as well as up-regulating glutamate transport itself [58,82]. Although cell-surface glutamate receptors are also expressed in astroglia, the use of selective receptor agonists and antagonists has excluded their involvement in these processes [58,81,82]. Treating cultured astrocytes with increased extracellular glutamate rapidly increased the V_{max} for glutamate transport, a process which may [58] or may not [82] be associated with increased cell-surface EAAT1 abundance. This effect was characterized by an EC_{50} for external glutamate of approx. 40 μM [58], a value of similar order to the K_m for glutamate transport by EAAT1. Increasing extracellular glutamate also activated the p42/44 MAPK pathway in a dose-dependent manner in astrocytes [81]. Other substrates for both EAAT1 and EAAT2 were also capable of mimicking the effects of increased glutamate, although the EAAT2 selective antagonist dihydrokainate could neither suppress the increased glutamate transport [58] nor the enhanced MAPK signalling [81]. Duan et al. [58] also demonstrated that transport-associated changes in cell volume, Na^+ and K^+ content, and membrane potential could not mimic the up-regulation of glutamate transport elicited by glutamate. Furthermore, γ-aminobutyric acid (GABA), which is transported into astrocytes by a mechanism of net stoichiometry similar to that of glutamate, did not increase glutamate transport capacity. These results suggest that the EAAT1 transporter participates in the activation of glial MAPK signalling in response to extracellular glutamate. Such a mechanism may help sensitize astrocytes to extracellular glutamate availability. Whether activation of the MAPK pathway directly regulates the cell-surface expression of EAAT1 has not been investigated, but, if it proves to be the case, it implicates EAAT1 as both a sensor and an effector in a process which could improve the efficiency of glutamate clearance at different levels of glutamatergic activity. This process may be analogous to a MAPK-dependent signal transduction cascade which is reported to upregulate cell surface glucose transporter expression on exposure to hyperglycaemia [83,84]. In the latter study, the authors isolated a specific region in the C-terminus of GLUT1 which may be involved in transporter-initiated signalling [83], although no recognizably similar motif is present in EAAT1.

Amino acid signal initiation through interactions between transporters and associated proteins

Protein–protein interactions are likely to be of great importance if amino acid transporters are capable of initiating signalling. A variety of proteins are reported to interact with amino acid transporters, including LIM (Lin-11, Isl-1 and Mec-3) domain proteins [85–87], heat-shock proteins (HSP) [88], cytoskeletal proteins [89,90] and integrins [91]. These interacting proteins have been implicated in diverse cellular functions, including the trafficking, localization and regulation of membrane transporters, as well as cell signalling [86,87,91]. Since many amino acid transporters have only recently been cloned, it is plausible that numerous other protein–protein interactions exist which have yet to be established. Nevertheless, we should point out that there is presently no direct evidence to show that the binding of amino acid substrates to EAAT1, SAT2 or other transporters of nutritionally important amino acids regulates direct interactions of transporters with other proteins or the activity of transporter-associated signalling molecules. As such, evidence supporting the hypothesis that these transporters may function as amino-acid-sensitive receptor molecules is indirect and limited. However, recent work studying GABA transporter 1 (GAT1) for the neurotransmitter amino acid GABA (outlined immediately below) has demonstrated that this hypothesis is not without foundation. We will then briefly discuss some of the transporter-associated proteins that are most likely to play a role in signal transduction.

GABA regulates GAT1–syntaxin 1A interactions

Substrate-induced changes in transporter activity are most evident in re-uptake systems for neurotransmitter amino acids such as GABA, glycine and glutamate, and may provide a co-ordinating link between processes of transmitter release and re-uptake [92]. The neurotransmitter transporter family includes transporters selective for noradrenaline ('norepinephrine'), 5-hydroxytryptamine ('serotonin') and taurine as well as amino acids, including GABA and glycine. GAT1 is one of several transporters and channels regulated by syntaxin 1A, a component of the synaptic-vesicle docking and fusion apparatus. Direct interaction between the N-terminal cytoplasmic domain of GAT1 and syntaxin 1A decreases substrate flux through the transporter [93]. GAT1 substrates increase transport rate by affecting GAT1–syntaxin 1A interactions; this appears to provide a mechanism by which to increase GABA transport when synaptic GABA

concentrations become elevated [94]. GAT1 substrates also enable tyrosine phosphorylation of the transporter, reducing its rate of internalization from the plasma membrane and hence increasing its overall expression at the cell surface [95]. This is the first demonstration that substrate–amino-acid-transporter interactions alter the accessibility/interaction of a mammalian transporter to/with classical signalling machinery. Identification of proteins that bind in a phosphorylation-dependent manner to GAT1 and kinases that phosphorylate GAT1 may highlight novel mechanisms for cellular GABA action.

SLC7 amino acid permeases and their interacting proteins

The SLC7 family of mammalian amino acid transporters comprises transporters for cationic (CAT1–3), neutral (LAT1–2, asc1) and both cationic and neutral amino acids [y+LAT 1–2, b(o,+)AT] [96]. SLC7 transporters belong to the APC transporter superfamily, alongside the yeast Ssy1p amino acid sensor considered in a previous section of this review. Many SLC7 transporters, including the System L amino acid transporters LAT1 and LAT2, require the presence of a glycoprotein (in this case CD98/4F2hc) to form a functional holotransporter in mammalian cells [50]. CD98 is an early T-cell activation antigen [97] which causes malignant transformation when overexpressed in NIH3T3 cells [98] and has also been implicated both in integrin signalling and regulation of cell–substratum interactions [91]. Furthermore, there is considerable evidence for the overexpression of both CD98 and its associated light chain in tumours [99] and leukaemia isolates [100]. LAT1 has been suggested to act as an 'environmental sensor' of amino acid availability [60] and this clearly may be facilitated by association with CD98.

Interactions with LIM-domain proteins

Ajuba and Hic-5 (hydrogen peroxide-inducible clone-5) are proteins containing LIM domains which have recently been shown to associate with EAAT2 [86] and the dopamine transporter DAT [87] respectively. LIM domains [101] are typically composed of two zinc-finger domains, which interact by virtue of their inherent hydrophobicity. Ajuba and Hic-5 are members of the same subfamily of LIM-domain proteins, containing several C-terminal LIM repeats and distinct N-termini. Ajuba also contains two Src homology 3 (SH3) recognition motifs and a nuclear export sequence and associates, via non-LIM regions, with the SH3-domain containing protein Grb-2, both *in vitro* and *in vivo* [102]. This interaction may lead to enhanced signalling via the classical p42/44 MAPK pathway. Ajuba has also been implicated in transcriptional regulation [103], cell proliferation and differentiation [104] and has been shown to redistribute in response to extracellular stimuli [104]. The functional relevance of the Ajuba–EAAT2 association is uncertain, since co-expression of Ajuba and EAAT2 in COS cells neither affects the V_{max} nor the K_m for EAAT2 activity [86]. Although it has not yet been tested, it seems plausible that the interaction between EAAT2 and Ajuba may regulate Ajuba and its downstream effectors. Unlike its close relative EAAT1, there is currently no evidence to suggest that EAAT2 is involved in modulating cellular signalling or gene expression; however, its interaction with a protein with diverse (albeit poorly defined) functions makes such possibilities seem likely. Ajuba may alternatively function as a link between EAAT2 and cytoskeletal proteins, helping to maintain the transporter in close proximity to the synaptic cleft [101]. The association of Hic-5 with DAT leads to reduced DAT activity, which occurs principally through a reduction in the plasma-membrane abundance of DAT [87]. Unlike the Ajuba–EAAT2 association, the binding of DAT to Hic-5 is mediated by LIM domains, although the subcellular redistribution of DAT may require the non-LIM domains of Hic-5. Hic-5 had previously been shown to associate with non-receptor tyrosine kinases [105,106] as part of focal-adhesion complexes [107], suggesting DAT co-localization may be of importance to intracellular signalling.

Interactions with glutamate-transporter-associated proteins (GTRAPs)

In NBL-1 cells an unidentified regulatory glycoprotein is associated with increased EAAT3 activity [59]. It is noteworthy that some GTRAPs (e.g. GTRAP41 and GTRAP48) can modulate glutamate transporter activity by primarily increasing transport V_{max}. GTRAP41 is thought to be involved in anchoring the EAAT4 glutamate transporter to the actin cytoskeleton, whereas GTRAP48 interacts with molecules involved in G-protein signalling. Thus it is conceivable that changes in glutamate transporter activity may trigger intracellular signalling via interacting proteins of this or analogous type [108]. Conversely, another GTRAP protein (GTRAP3-18), which associates with the intracellular C-terminal domain of EAAT3, has been reported to inhibit glutamate transport activity [109]. The mechanisms that regulate GTRAP/transporter interactions remain poorly understood, but given that interaction of certain GTRAP proteins with their respective carriers can either stimulate or inhibit transport function, such interactions may be mechanistically important in the transduction of glutamate-evoked signals.

Interactions with cytoskeletal proteins and integrins

A protein network lying beneath the plasma membrane, composed of proteins such as fodrin and ankyrin, may function to link the microtubule, microfilament and intermediate-filament systems with the cell surface [110]. This network may therefore have important roles in signalling from integrins and focal adhesions, which potentially act as cell-volume sensors [111]. Partial purification demonstrated that the hepatic System A transporter closely associates with integrin α_3–integrin β_1 dimers [112]. Fodrin has been shown to associate with the CAT1 amino acid transporter in pulmonary artery endothelial cells [89] and also with hepatic System A transporter activity [90]. In the retina, the atypical variants GLYT1E (glycine transporter 1E) and GLYT1F of GLYT1 may associate with the ρ-subunit of the GABA(C) receptor [113], which, through a distinct region, also binds the microtubule-associated protein (MAP) 1B [114]. Disruption of the MAP1B–GABA(C) association results in a decreased EC_{50} for GABA at the GABA receptor, although alteration of glycine availability (and therefore GLYT1 transport) does not appear to affect retinal GABA signalling [115]. Co-localization of the receptor and transporter may help co-ordinate the convergence of glycinergic and GABAergic signalling downstream of the GABA receptor and/or interactions with the cytoskeleton.

As discussed previously, coupled amino acid transport has been implicated in the regulation of cell volume [73,116–121]. Amino acid transport is itself also very sensitive to aniso-osmolarity [16,17,46,59,77,122–124]. Given the links between the cytoskeleton, integrins and amino acid transporters and the dependence of volume-sensitive signalling on the cytoskeleton [77,111], the co-localization of amino acid transporters with volume-sensitive signalling molecules may lead to local microdomains of the plasma membrane that are particularly responsive to cellular swelling and/or shrinkage. Many of the cellular proteins involved in regulatory changes of cell physiology to

altered osmolarity are plasma-membrane transporters, including channels, pumps and transporters of amino acids and other osmolytes [16,17,116,125,126]; therefore the co-localization of these proteins with the volume-sensitive machinery may also allow for rapid alteration of transporter activity during aniso-osmotic conditions (as considered in the next section).

AMINO ACID SENSING AS AN INDIRECT CONSEQUENCE OF THE AMINO-ACID-TRANSPORT MECHANISM

Extracellular and intracellular conditions can vary considerably with respect to each other. Membrane proteins lie at the interface between these two compartments and may therefore be capable of relaying signals relating extracellular conditions to the cell interior. The activity of specific amino acid transporters results in changes in cell volume, transmembrane electrical potential difference and/or pH, which may be detectable by cell sensor mechanisms or may even be of sufficient magnitude to initiate or suppress intracellular signals themselves. Furthermore, such changes in cell physiology may feed back to modulate the transport processes themselves, with important implications both for cellular metabolism and the ability of transporters to contribute to nutrient signalling.

Cell volume change

One indirect effect of certain transport mechanisms is cellular swelling [12,16,27,73,116,119,121,127–129]. The cellular accumulation of organic osmolytes is not particularly cytotoxic, thus cells may accumulate high levels of amino acids, polyols and methylamines to counteract extracellular hypertonicity. Osmolyte accumulation draws water molecules into the cell and may partially restore cellular volume during conditions that favour cellular shrinkage. Accumulative transport of amino acids may also lead to cell swelling during isotonic conditions; for example, transport via System A and System X^-_{AG}, which (in terms of bulk flow) both lead to the cellular accumulation of one amino acid and one inorganic cation per transport cycle, are associated with the passive cellular accumulation of water molecules. This type of phenomenon leads to swelling of both liver and muscle cells in response to an increase in extracellular concentration of amino acids such as glutamine (an excellent substrate of Systems A and N). This cell swelling in response to increased amino acid loading has general anabolic effects such as stimulation of protein and glycogen synthesis and suppression of protein breakdown. In muscle cells at least, the signalling mechanisms activated in these circumstances appear to be the same as those activated by hypotonic cell swelling, suggesting that the anabolic stimulus may be swelling itself. There is evidence in both muscle [111] and liver [16] that integrin (adhesion)-dependent mechanisms are involved in sensing cell-volume change and initiating downstream signals. In muscle at least, the activated signalling pathways include the mTOR pathway [28]. The work of Bevington et al. [52] suggests that the anabolic role of intramuscular glutamine may be specifically enhanced by amino acid transport through System A in L6 skeletal-muscle myotubes. This work also indicated a role for System A activity in maintaining the intracellular-free-pool sizes of branched-chain amino acids, probably through maintaining their tertiary active transport [52]. Increasing the intracellular concentration of branched chain amino acids in this way, as a consequence of enhanced glutamine uptake through secondary active transporters such as System A, is likely to directly activate mTOR signalling (as described in the next subsection), this process, rather than cell swelling, may be the major mechanism by which cellular glutamine uptake appears to activate the mTOR pathway in skeletal muscle. Previous work suggested that transport through System N^m (an amino acid transporter awaiting molecular identification), rather than System A, may be the major regulator of intracellular glutamine pool size in intact skeletal muscle [23,130,131]. Unfortunately, a transport function similar to System N^m has not been characterized in L6 myotubes for comparative study. The recent discovery that System A transporters are likely to have a higher concentrative capacity for amino acid substrates than System N isoforms [132] raises the possibility that the *in vivo* protein-sparing role of glutamine in muscle may be mediated through activity of System A (for which glutamine is also a substrate) rather than System N^m.

When amino acids are absent from the incubation medium, cells lose one mechanism for the stabilization of cellular volume, and amino acid starvation may therefore lead to cellular shrinkage [76]. As considered above, the induction of System A activity by amino acid deprivation may be at least partly a response to cellular shrinkage in certain cell types. Consistent with this proposal, the adaptive regulation of System A in these cells is mimicked by hypertonicity [73,78]. Both hypertonicity and amino acid deprivation increase System A expression through gene-expression-dependent mechanisms; this is related to activation of the p42/44 MAPK and JNK pathways in human fibroblasts [76]. Both amino acid deprivation and hypertonicity lead to the nuclear redistribution of the tonicity-sensitive enhancer binding protein ('TonEBP') and to the up-regulation of both System A activity and the sodium *myo*-inositol co-transporter ('SMIT') [125]. Tight control of System A by both regulated gene expression and *trans*-inhibition may, therefore, be a key mechanism for the regulation of both intracellular amino acid pool size and cell volume (particularly in cells lacking other Na^+-dependent amino acid transporters).

Transmembrane electrical potential difference

Stimulation of hormone secretion is a major role of a variety of amino acids that is of fundamental importance to the control of nutrient balance and disposal. Arginine-stimulated insulin release from pancreatic β-cells appears to result directly from membrane depolarization occurring due to its electrogenic transport (presumably through System y^+), since non-metabolizable arginine analogues [including some NO synthase (NOS) inhibitors] mimic this effect [133,134]; the resultant membrane depolarization stimulates Ca^{2+} influx via opening of voltage-gated channels, leading to increased insulin secretion by exocytosis (reviewed in [8,135]). Arginine also potentiates glucose-induced insulin secretion [133,134,136,137]. By contrast, the insulin secretagogue action of neutral amino acids may partially be explained through altered cellular ATP levels. Elevated ATP leads to the closing of specific plasma-membrane K^+ channels with a resultant membrane depolarization, which then triggers insulin secretion by the mechanism described above for arginine. Nevertheless, synthetic non-metabolizable amino acid analogues such as α-aminoisobutyric acid (AIB, a substrate of Systems A, ASC and L) also stimulate insulin secretion [9], raising the possibility that depolarizing effects of the electrogenic Na^+-coupled transport of AIB through System A may also help initiate insulin secretion. There is also some evidence that β-cell swelling (either in response to stimulation with hypotonic solutions or to isotonic solutions containing compounds that are rapidly transported into the cell) may stimulate insulin release [138,139]. As discussed in the previous subsection, amino acid transport may partially regulate cell volume. Therefore, the secretagogue action of certain amino acids may be a combined consequence

of transport-associated effects on membrane potential and cell volume, in addition to an increase in the intracellular abundance of metabolic products of amino acids (such as ATP).

Effects of pH change

The mechanism of certain amino acid transporters involves transmembrane movement of H^+ (or OH^-) ions [140,141] and will therefore lead to changes in intracellular pH, which may conceivably be of sufficient magnitude to result in modulation of intracellular processes. As such, prolonged cell exposure to low pH reduces the cellular capacity for concentrative glutamine uptake, thus reducing the free intracellular glutamine concentrations with knock-on effects on intracellular concentrations of essential amino acids such as leucine (for reasons described above) and cell functions such as protein turnover [142,143].

A potentially more important phenomenon is the pronounced sensitivity to pH changes within the physiological range exhibited by amino acid transport Systems A and N. Although not an amino-acid-sensing mechanism *per se*, this pH-sensitivity may have major pathophysiological consequences for cellular nutrient sensing. For example, Bevington et al. recently [52] sought to determine whether a single pH-sensitive transport activity might be responsible for the catabolic response to acidosis observed in skeletal muscle [144]. This group demonstrated that, in L6 myotubes, Me-AIB transport through the System A amino acid transporter (presumably through SAT2 [75,75]) was inhibited rapidly and selectively in a reversible fashion in response to a physiologically relevant extracellular acidosis (pH 7.1). In addition, exposing muscle cells to a saturating dose of Me-AIB to competitively inhibit System A transport at neutral pH induced an increase in cellular protein degradation that was comparable with that observed by incubating cells at pH 7.1 [52]. Furthermore, the responses to Me-AIB and acid were antagonistic to the known protein-stabilizing effect of the System A substrate glutamine [23,145–147]. These results all support the view that inactivation of System A may be the primary event initiating the cellular catabolic response to low pH.

Several recent reports have suggested a role for intracellular glutamate generation in glucose-induced insulin secretion [148–151], although other reports dispute such a role [152–154]. The molecular mechanisms involved in β-cell glutamate sensing are poorly defined, but this response may be mediated independently of the well-documented K_{ATP}–Ca^{2+} pathway, possibly by a pH-dependent mechanism as now described. Maechler and Wollheim [150] demonstrated that the response of β-cells to glutamate is inhibited in the presence of Evans Blue and bafilomycin – compounds that impair vesicular glutamate transport [155–158]. These results are suggestive of a role for transmembrane amino acid transport between specific intracellular compartments in regulating the exocytosis of insulin secretory vesicles. Three vesicular glutamate transporters (VGLUT1–VGLUT3) have been characterized in neuronal tissues, where they play a vital role in neurotransmitter release [155–165]. Of these transporters, VGLUT1 and VGLUT2 have been identified in both pancreatic α- and β-cells [166–168], whereas VGLUT3 mRNA is absent from the pancreas [156]. How vesicular glutamate accumulation may affect insulin secretion is presently uncertain, although changes in intravesicular pH have been implicated in vesicular fusion [169], and a similar mechanism may be envisaged for the maturation of insulin secretory vesicles, since glutamate accumulation via the VGLUT proteins is associated with intravesicular pH change [158,170]. The molecular mechanisms involved in the regulation of vesicular pH by glutamate are uncertain; however, these may involve transport associated H^+ movement and/or a role for vesicular glutamate as a counterion for H^+. Several other glutamate sensors are also localized to pancreatic β-cells, including both ionotropic [171] and metabotropic [172] glutamate receptors and a glutamate-sensitive protein phosphatase that has been implicated in the formation of malonyl-CoA [173] – a potential insulin secretagogue (reviewed in [8]).

AMINO ACID TRANSPORTERS AND INTRACELLULAR SENSORS OF AMINO ACID AVAILABILITY

Signalling pathways that are regulated by intracellular amino acid concentration are intrinsically linked to amino acid transporter activity as well as to intracellular amino acid metabolism. The intracellular concentration of any given amino acid is governed by the rates of amino acid influx and efflux, protein synthesis, protein degradation and aminoacyl-tRNA production, plus amino acid catabolism and biosynthesis. Increased extracellular amino acid concentration may lead to increased intracellular amino acid concentration by virtue of concentrative or equilibrative transmembrane transport. Evidence exists demonstrating a role for intracellular amino acid sensing in GCN2 and mTOR signalling as well as the regulation of NO synthesis. Furthermore, thyroid hormones (essentially large iodinated amino acids which must traverse the plasma membrane to exert cellular effects through nuclear receptors) are substrates for the System L amino acid transporter, which appears to enhance the availability of thyroid hormone to nuclear thyroid receptors in certain cell types [174].

Amino acids regulate a number of intracellular enzymes, and these may play a role in nutrient sensing (Figure 1). Examples include the (presently uncloned) glutamate-activated protein phosphatase-2A [173,175] and mitochondrial glutamate dehydrogenase, which is allosterically activated by leucine [176]. The accumulation of uncharged tRNA molecules may occur following prolonged amino acid deprivation, and these molecules may also be sensed within the cell. (6-Phosphofructo-1-kinase (PFK1) is inhibited by uncharged tRNA *in vitro* [177], potentially leading to a reduction in intracellular fructose-1,6-bisphosphate {an activator of the translation factor eIF2B (eukaryotic initiation factor 2B) ([178,179]}.) In yeast, the protein kinase GCN2 is activated by uncharged tRNA and inactivates the translation factor eIF2α by phosphorylation. A mammalian homologue of GCN2 has been cloned, and it functions in the response to amino acid starvation [180]; however, it is presently uncertain whether mammalian GCN2 is activated by uncharged tRNA.

Amino acid transport and mTOR signalling

mTOR signals to a variety of molecules that regulate translation. These include translational repressors, such as the 4E-binding proteins (4E-BP) and stimulators of translation, such as ribosomal protein S6 kinase (S6K), which phosphorylates a ribosomal subunit (S6) and has been implicated in the regulation of translation elongation via eukaryotic elongation factor 2 ('eEF2') [181]. Elevated amino acid availability signals via mTOR and culminates in increased cellular translation rates.

With respect to TOR signalling, many studies have focused on the potent stimulatory effects of leucine, and several lines of research have implicated the intracellular accumulation of this amino acid in signal initiation [53,182]. Leucine is transported into most mammalian cells by System L, but, in mammalian studies, mTOR signalling is typically much more sensitive to

leucine than to other closely related System L substrates such as valine and isoleucine [41,183,184]. Using a combination of amino acid microinjection and System L transporter overexpression techniques in *Xenopus laevis* oocytes, our group has been able to demonstrate that only under circumstances where amino acid concentrations are increased within the cytoplasm do they activate the TOR pathway [182]. Overexpression of System L confers sensitivity to extracellular amino acids only inasmuch as it facilitates their delivery to the cytosol. Such evidence indicates an intracellular localization for an amino acid sensor upstream of the TOR pathway in *X. laevis* oocytes, but also highlights the importance of plasma-membrane transporters as conduits for amino acid delivery to the intracellular receptor mechanism. Our evidence is also consistent both with the proposal that the size of the intracellular free amino acid pool regulates mTOR signalling (given that aminoacyl-tRNA composition may not be significantly affected by short-term amino acid deprivation [22]) and the demonstration that competitive inhibitors of amino acid transport inhibit amino-acid-induced activation of mTOR in human Jurkat cells [185]. Injected amino acids activating the TOR pathway in *X. laevis* oocytes included leucine, tryptophan, phenylalanine, arginine and lysine, whereas glutamine, glutamate, proline and alanine were relatively ineffective. Nevertheless, amino acids such as glutamine may influence mTOR signalling *in vivo* through processes such as tertiary active transport (as described above). Microinjection of leucinol and tryptophanol also stimulated TOR signalling in this system, indicating that the *X. laevis* sensor may additionally be responsive to a range of amino acid metabolites [182]. The ligand-sensitivity of TOR signalling in microinjected *Xenopus* oocytes is different from that observed in mammalian cells, where, in particular, the amino acid alcohols are at best poor stimulants of TOR signalling. The System L transporter is up-regulated by activation of the mTOR pathway in mammalian cells, providing an increased capacity for essential amino acid supply to support cell growth [186]; this may also help to modulate cell growth in relation to extracellular nutrient supply.

Amino acid metabolites and nutrient signalling

Amino acids are utilized as metabolic fuels for the formation of a variety of metabolites, which may be important in nutrient signalling. For example, ATP generated following amino acid metabolism may enhance both mTOR signalling and insulin secretion – the former due to the utilization of ATP as a kinase substrate and the latter as a result of ATP-sensitive membrane depolarization (reviewed in [187]). mTOR signalling is very sensitive to intracellular ATP [22] and is also inhibited by treatments which mimic high intracellular AMP [22,188–192]. The effect of AMP on mTOR signalling appears to be mediated through the AMP-activated protein kinase (AMPK), since the compound 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside, which stimulates AMPK signalling, can mimic this response [188–191]. Amino acid alcohols have actually been shown to inhibit amino acid signalling through the mTOR pathway in Jurkat cells [185], although not in adipocytes [193]. The inhibition exerted by amino acid alcohols in some mammalian cell-culture systems may stem from their ability to inhibit tRNA charging and thus activate GCN2, which would result in a suppression of mRNA translation [185]. However, uncharged tRNAs may also inhibit glycolysis through the inhibition of PFK1 (as mentioned above). Amino acid alcohols may therefore reduce the cellular ATP/AMP ratio and thus inhibit signalling to S6K, via the activation of AMPK. However, AMPK has been shown to stimulate cardiac 6-phosphofructo-2-kinase (PFK2; a major regulator of glycolytic flux) by phosphorylation and putative AMPK phosphorylation sites also exist within both placental and inducible isoforms of PFK2 (reviewed in [194]). The metabolic effects of uncharged tRNAs may therefore be cancelled out in cell lines expressing AMPK-sensitive PFK2 isoforms. However, in cells expressing PFK2 isoforms that are not sensitive to AMPK (such as skeletal muscle and liver [194]), uncharged tRNAs may inhibit PFK1 without any compensatory activation of PFK2 by AMPK, thus reducing glycolysis. The consequential effects of glycolytic inhibition may include the inhibition of mTOR signalling.

The induction of System X^-_{AG} by amino acid deprivation in NBL-1 cells is repressed by L-glutamate and L-aspartate, substrates for EAAT3, although certain specific non-substrate amino acids (notably alanine, glutamine and asparagine, but not Me-AIB or leucine) also have a repressive effect [59,195]. These EAAT3-repressive non-substrates are amino acids that may undergo rapid intracellular metabolic conversions to yield glutamate and aspartate. To test whether the intracellular concentration of glutamate may regulate EAAT3, Nicholson and McGivan [195] utilized a combination of glutamine deprivation and amino-oxyacetate (a transaminase inhibitor). This treatment induced a significant decrease in intracellular glutamate and was associated with elevated aspartate transport activity. It therefore appears plausible that the intracellular concentration of anionic amino acids regulates EAAT3 activity. The fact that D-glutamate is a relatively poor EAAT3 substrate, but has a potent repressive effect, does not exclude the possibility of an extracellular sensing component [54,195] or the possibility that EAAT3 (which may be able to bind to amino acids on both the cytoplasmic and extracellular surface) may be a functional amino acid sensor. Intracellular transamination is a mechanism by which glutamine accumulated in a cell may be used to convert intracellular oxo acids into amino acids [196] rather than help drive the counter-exchange of System L substrates such as leucine [49]. The former route may be a major mechanism for hepatic accumulation of branched-chain amino acids (leucine, isoleucine and valine) and thus should contribute to activation of the mTOR pathway.

NO as a nutrient-responsive signal

NO is a second messenger with multitudinous roles, conceivably including nutrient-induced signalling. NO is formed from the conditionally essential amino acid arginine and, although intracellular arginine concentrations may be high enough to support NO synthesis, in many cells sustained NO formation requires an extracellular arginine source. Extracellular arginine may be channelled to NOS via plasma-membrane transporters, and NO synthesis may be enhanced in many cells by the co-ordinate up-regulation of NOS isoforms and cationic amino acid transporters [197]. Indeed, there is evidence for the intracaveolar co-localization of CAT1 with an endothelial NOS (eNOS) isoform [198], and this may provide a novel mechanism for the regulation of NO production by increased arginine influx, either through elevation of extracellular arginine or by increasing arginine transporter activity [197,199]. The concept that eNOS may act as a transducing sensor of arginine availability in endothelial cells is strengthened by the observation that disruption of the actin cytoskeleton, which suppresses arginine uptake, also leads to an attendant loss in NO generation. This diminution in NO production occurs without any change in cellular eNOS expression or activity, suggesting that CAT1 transport is a key determinant of NO synthesis and signalling [200].

AMINO ACID TRANSPORTERS AND EXTRACELLULAR AMINO ACID SENSORS

Extracellular amino acid receptors have been identified in numerous eukaryotic cells and include, for example, the neuronal receptors for glutamate [48] and glycine [201] as well as the G-protein-coupled taste receptor heterodimer T1R1–T1R3 [202]. This taste receptor is believed to mediate a sweet taste and is sensitive to a broad range of L-amino acids, although the precise ligand specificity appears to be species-dependent [202]. The leucine oligomer-based membrane-impermeant molecule Leu8-MAP (Leu_8-Lys_4-Lys_2-Lys-βAla) [203] and the leucine analogue isovaleryl-L-carnitine [204] (discussed in [14]) both efficiently inhibit hepatic macroautophagy in a similar manner to leucine itself and Leu8-MAP was therefore suggested to associate with a membrane bound 'leucine' receptor with regulatory control over proteolysis. This putative leucine receptor does not appear to interact with the mTOR pathway [14,193]. Amino acid availability may additionally generate or regulate signal transduction through other extracellular receptors such as the calcium receptor, CaR, a close homologue of metabotropic glutamate receptors and $GABA_B$ receptors. Work by Conigrave et al. [205] has demonstrated both that CaR can be stimulated by extracellular amino acids (particularly aromatic amino acids) in the presence of millimolar concentrations of extracellular Ca^{2+} and also that amino acids sensitize CaR to extracellular Ca^{2+} (reviewed in [206]). Furthermore, leucine (also alanine and glycine) has been shown to modulate purinergic G-protein-coupled receptor (P2Y) signalling, thereby providing a possible alternative type of mechanism through which cell-surface receptors might initiate or modulate some of the *in vivo* responses to leucine [205].

We described above the association of amino acid transporters with other proteins, including surface receptors. The proximity of transporters to receptors may facilitate fine-tuning of receptor signalling at the cell surface – this is already well-recognized for synaptic neurotransmission (as described below), but may conceivably have more general relevance. Glutamate is released from presynaptic neurons and stimulates postsynaptic glutamate receptors, ultimately leading to postsynaptic neurotransmission. Unlike many other neurotransmitters (such as acetylcholine), glutamate is not metabolized within the synaptic cleft, and uptake of glutamate into both the neurons and the surrounding astrocytes by the EAATs is of great importance in attenuating glutamate signalling. These transporters maintain a low synaptic concentration of glutamate and serve to rapidly clear synaptic glutamate upon its release from the presynaptic neuron. Under normal circumstances, extracellular glutamate levels are carefully regulated at the synapse by both astrocytic (EAAT1 and EAAT2) and neuronal (EAAT3) transporters [207,207]. Inefficient clearance of synaptic glutamate may be neurotoxic, owing to prolonged hyperactivation of postsynaptic glutamate receptors, and has been implicated in the development of ALS [24]. Indeed, certain isolates of familial ALS are associated with poor glutamate clearance by EAAT2, demonstrating the importance of astrocytic transporters in neuronal health [208].

CONCLUSION

We have brought together work from a variety of research areas in an attempt to present as a coherent concept the idea that transporters in the plasma membrane provide a potentially important (and generally overlooked) system for governing cellular and organismal responses to amino acids. Although the concept of solute transporters at the cell surface acting as 'environmental sensors' is perhaps an obvious one, there remains relatively little direct evidence that it occurs generally in cells of higher animals, especially with regard to sensing of nutrient availability. With specific regard to amino acids, many of the best-studied examples relate to their detection as part of the process of neurotransmission, where it is becoming clear that transporters may contribute substantially to mechanisms of neuromodulation at synapses. The transporters involved here belong to the same gene families (or in some cases are the same gene products) as those expressed on other cell types; therefore it seems reasonable to speculate that the sensing and signalling functions ascribed to certain amino acid transporters in the central nervous system may have more widespread distribution and relevance. Further investigation is needed to fully clarify these issues, with a major long-term goal of developing strategies to modulate sensor activation therapeutically as a means to enhance nutrient responsiveness in catabolic disease or selectively reduce it in tumours.

Note added in proof (received 27 May 2003)

Following the publication of a recent review [217], the nomenclature for the System A and System N family of sodium-coupled neutral-amino-acid transporters has changed. SAT1, SAT2, SN1, SAT3 and SN2, which are mentioned in Table 1, are subsequently to be referred to as SNAT1–5 respectively.

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