Reviews

The regulation of neutral amino acid transport by amino acid availability in animal cells

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Animal cells regulate the activities of neutral amino acid transport Systems A and L to keep the intracellular supply of amino acids relatively constant. Transport System A activity increases dramatically in response to starvation of all the amino acids. Transport System L activity increases in response to starvation of a single substrate such as leucine. The mechanism of regulation appears to be different for Systems A and L.

In animal cells, as well as in microorganisms, the intracellular levels of most amino acids are regulated to meet the nutritional requirements of the cells. The amino acid levels are maintained by balancing the uptake of amino acids from the extracellular medium, and the biosynthesis of certain of the amino acids, with the needs of the cell for amino acids for protein synthesis and energy metabolism. The uptake of the neutral amino acids in animal tissues is carried out by several distinct transport systems, which differ in their reactivities with substrates, ions and inhibitors. Although the transport systems have been examined kinetically in a great many tissues and cell types, only recently have these studies included the characterization of the regulation of transport. Transport activity in animal cells is affected, for example, by the cell density[1–4], the position in the cell cycle[4] and viral transformation[1,3,5]. This review, however, will be restricted to some of the recent studies on the regulation of neutral amino acid transport by changes in the availability of amino acids from the extracellular medium.

Major neutral amino acid transport systems

The uptake of neutral amino acids by animal cells is divided among a few transport systems that have overlapping substrate specificities. The characterization of the transport systems has been aided by the use of a number of non-metabolizable amino acid analogs that are largely restricted in their uptake to single transport systems. Neutral amino acid transport Systems A and L were first identified in the Ehrlich ascites tumor cell[6]. System A is most effective for amino acids having short, polar, or linear side chains, such as alanine, glycine, and the non-metabolizable analog 2-aminoisobutyric acid (AIB) and its *N*-methylated derivative, 2-methylaminoisobutyric acid (MeAIB). Transport by System A is sodium ion-dependent and is greatly reduced at lowered extracellular pH. Uptake of amino acids by System A is often reduced by the presence of intracellular substrates of this system, the phenomenon of *trans*-inhibition[7].

In contrast, System L is most effective for branched-chain and aromatic amino acids, such as leucine, isoleucine, valine, phenylalanine, and the non-metabolizable analog 2-aminobicyclo-[2,2,1]-heptane-2-carboxylic acid (BCH). Transport by System L is sodium ion-independent, and in some cases it is even stimulated by lowered extracellular pH. Uptake of amino acids by System L is increased by the presence of intracellular substrates of this system, a phenomenon known as *trans*-stimulation[7]. Amino acid transport systems corresponding to Systems A and L have been described in a wide variety of animal cell types, including cells of avian and mammalian origin (for reviews, see Refs 8 and 9).

A second sodium-dependent transport system has also been characterized in the Ehrlich cell[10]. This system, which seemed to transport alanine, serine, and cysteine in Ehrlich cells, was designated System ASC. In addition to its substrate preference, System ASC is distinguished from System A on the basis of its relative insensitivity to pH, higher stereospecificity, intolerance of *N*-methylated substrates and ability to be *trans*-stimulated by intracellular amino

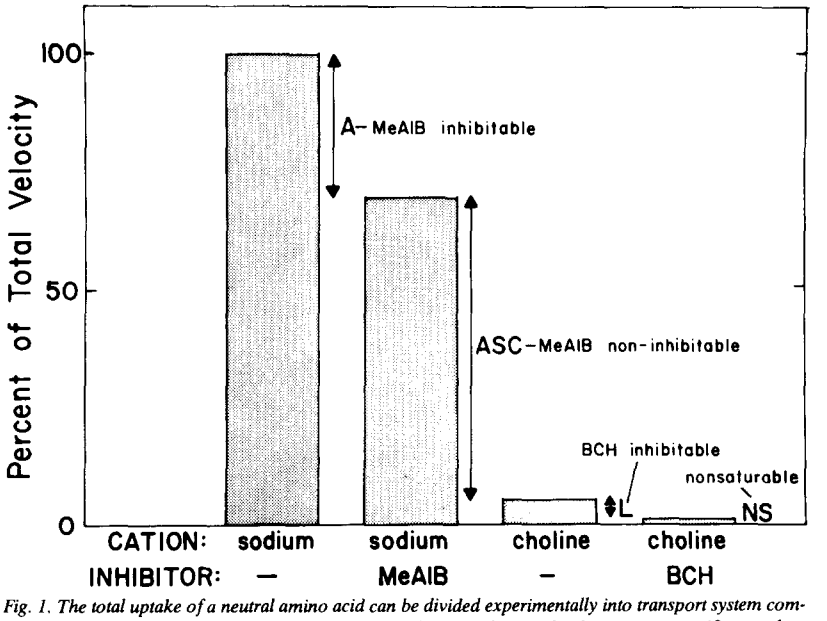

Fig. 1. The total uptake of a neutral amino acid can be divided experimentally into transport system components using sodium-containing and sodium-free media and the system-specific analogs 2-methylaminoisobutyric acid (MeAIB) and 2-aminobicyclo-[2,2,1]-heptane-2-carboxylic acid (BCH). The System A component can be taken as the portion of the total uptake that is inhibited by excess MeAIB. The System ASC component can be taken as the sodium-dependent uptake that is not inhibited by excess MeAIB. The System L component can be taken as the sodium-independent uptake that is inhibited by excess BCH. The sodium-independent uptake that remains in the presence of excess BCH can be defined as the non-saturable (NS) component (see also Ref. 15).

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acids. Although System ASC is much less prominent than System A in the Ehrlich cell, it is the predominant sodium-dependent transport system in the rabbit reticulocyte[11] and pigeon erythrocyte[12] – cells in which System A activity is greatly reduced and difficult to evaluate. System ASC is also the most prominent sodium-dependent amino acid transport system in the rat hepatocyte[13], cultured human fibroblast[14] and Chinese hamster ovary cell[15,16]. The lack of a generally acceptable model amino acid for System ASC complicates the characterization of this system in the presence of the other neutral amino acid transport systems. In most cases, System ASC activity is dependent on sodium but not inhibited by MeAIB[15,16].

Fig. 1 illustrates the way in which the total uptake of an amino acid can be experimentally divided into components of uptake by transport Systems A, ASC and L. Table I is a summary of the characteristics of the neutral amino acid transport Systems A, ASC, and L, present in animal cells.

TABLE I. Characteristics of the major neutral amino acid transport systems in animal cells

	System A	System ASC	System L
1. Cation-dependence	Sodium-dependent	Sodium-dependent	Sodium-independent
2. Substrate reactivity	Most, esp. Ala, Gly, Pro, AIB	Ala, Ser, Cys[a]	Most, esp. Leu, Ile, Val, Phe
3. Specific substrate	MeAIB	None[b]	BCH
4. *Trans* effects	*Trans*-inhibited	*Trans*-stimulated	*Trans*-stimulated
5. Effect of low pH	Inhibited	Variable	Stimulated
6. Stereospecificity	Moderate	High	Moderate
7. Regulation by amino acid limitation	Yes	No	Yes[c]
8. Hormonal regulation	Yes	No	No
9. Cell distribution	Most, not erythrocyte, reticulocyte	Ubiquitous?	Ubiquitous?

[a] A broader substrate reactivity (including Thr, Leu, Ile, and Phe) has been observed in Chinese hamster ovary cells[15,16] and rat hepatocytes[17].

[b] Cys has been proposed to be a specific substrate in rat hepatocytes and intestinal epithelium[18], and Thr has been proposed to be specific in the hepatoma cell line HTC[19].

[c] Enhanced activity is caused by leucine limitation in Chinese hamster ovary cells[27,28].

Regulation of System A by amino acid availability

Riggs and Pan, using immature rat uterus preparations[20], and Guidotti and co-workers, using chick embryo heart cells[21], first observed that incubation of certain tissues in amino acid-free medium leads to an increase in neutral amino acid transport activity. This response has been referred to as adaptive regulation or starvation-induced transport enhancement. The increased transport activity has been identified with System A on the basis of its sodium ion-dependence and pH sensitivity, and by inhibition analyses[20,21]. The addition of System A amino acids to the medium prevents the enhancement of transport activity, but typical substrates of System L do not[21]. Individual System A amino acids suppress the System A transport increase in the absence of all other amino acids, and the analog AIB is particularly effective[21,22]. The starvation-induced transport is generally accompanied by an increase in the V_{max} of uptake of amino acids by System A with no significant change in the K_m of uptake[20,21], consistent with an increase in the number of active System A transport carriers. Because the increased transport activity can still be observed in cells depleted of amino acids prior to uptake measurements, release from *trans*-inhibition can be ruled out as a basis for the adaptive response to amino acid deprivation[21].

An examination of the relationship between amino acid transport and the supply of amino acids showed that System A activity varies inversely with the concentration of System A amino acids in the culture medium[23]. Higher levels of substrates of System A in the growth medium thus lead to lower System A activities. The regulation of System A transport activity by amino acid supply has now been described in many tissue and cell types[9] including cells in culture[15,23–25].

The increase in System A activity as a result of starvation of amino acids apparently requires synthesis of both RNA and protein. Inhibitors both of transcription and translation have been shown to prevent the transport enhancement in several cell types[9,15,20,21,24–26]. Guidotti and co-workers have concluded, from their studies using inhibitors of macromolecular synthesis, that System A transport activity is regulated by a repression-derepression mechanism acting at the transcriptional level[26]. The reversal of the starvation-induced System A enhancement by re-feeding with amino acids also requires active RNA and protein synthesis[26]. The decay of the elevated uptake of basal levels is accelerated by the addition of System A amino acids. Guidotti and colleagues have proposed a model for System A regulation involving a factor that degrades or inactivates the transport mechanism following re-feeding of starved cells with System A amino acids[26]. These same workers have suggested that the adaptive changes in System A activity act to compensate for the variations in the availability of external amino acids so that nearly constant accumulation of amino acids is maintained over a broad range of extracellular concentrations[9].

Regulation of System L by leucine availability

System L transport activity is also regulated in animal cells. Many of the early studies using several cell types showed that System L activity does not increase in response to complete starvation of amino acids as System A does[9]. In all cases the activity of System L was either unchanged or slightly decreased following amino acid starvation. Adaptive increases in System L transport can, however, be easily observed in a temperature-sensitive leucyl-tRNA synthetase mutant of the Chinese hamster ovary line (CHO-*ts*Hl), after growth at marginally permissive temperatures[27,28]. At these temperatures the cells become starved for leucine because of their inability to form leucyl-tRNA.

The CHO-*ts*Hl cell line has a temperature-sensitive leucyl-tRNA synthetase and grows normally at 34°C but cannot grow at 39°C. When CHO-*ts*Hl cells are incubated at 38°C, a temperature at which the leucyl-tRNA synthetase is partially inactivated, the transport activity for leucine increases two- to three-fold[27,28]. This temperature-dependent increase in leucine transport has been identified with System L on the basis of its sodium ion-independence, substrate reactivity and inhibition by the System L-specific analog, BCH (Ref. 28). This transport enhancement is reflected by an increase in the V_{max} of leucine uptake with no change in its K_m of uptake, consistent with an increase in the number of System L transport carriers[28].

The temperature-dependent elevation of System L activity in CHO-*ts*Hl cells cannot be explained by a *trans*-stimulation of System L by intracellular amino acids because cells depleted of amino acids prior to uptake measurements still show increased transport[28]. The transport enhancement can be prevented by cycloheximide but not by actinomycin D, which suggests that System

L may be regulated at the translational level[28].

Increases in System L activity can also be observed in non-mutant Chinese hamster ovary cells, although it is necessary to incubate the cells in media containing very low concentrations of leucine (less than 10 μM) and normal levels of the other amino acids[28]. Starving normal Chinese hamster ovary cells of isoleucine, valine or phenylalanine, other System L substrates, also leads to increases in System L activity although these increases are only about one-half of that following leucine starvation (Lobaton, Moreno and Oxender, unpublished results). Thus, starving Chinese hamster ovary cells of System L amino acids, whether by incubating a temperature-sensitive amino-acyl-tRNA synthetase mutant at an elevated temperature, or by incubating non-mutant cells in very low concentrations of System L amino acids, leads to increased amino acid transport activity by System L. The regulation of the activity of transport System L appears, therefore, to reflect the availability of System L amino acids for protein synthesis; conceivably the signal for this regulation may be the ratio of charged to uncharged tRNA, rather than simply the size of the intracellular pool of System L amino acids.

Regulation of Systems A and L

Although the amino acid starvation-induced regulation of System A and the leucine-dependent regulation of System L, in animal cells, are both adaptive responses leading to alterations in the activity of a specific amino acid transport system, significant differences exist between the two regulatory mechanisms. First, System A derepression can be observed only after limitation of all System A amino acids, but increased System L activity results from the deprivation of but a single System L amino acid such as leucine. Second, whereas System A activity rises gradually for 12 to 20 hours of amino acid starvation[23,25], the maximum System L transport enhancement occurs within the first 4 to 6 hours of leucine limitation[28]. Third, the increased activity of System A is slowly reversed upon amino acid re-feeding, requiring 8 or more hours[24,26], but the reversal of enhancement of System L activity is complete within 6 hours after shifting CHO-*ts*HI cells from an elevated temperature back to 34°C (Ref. 27 and Shotwell, unpublished results). Finally, whereas the derepression of System A is prevented by inhibitors both of transcription and translation[9,20,21,24–26], the System L enhancement in CHO-*ts*HI cells does not appear to be dependent upon active RNA synthesis for the first 4 hours[28]. Accordingly, it has been suggested tentatively that different sites of control exist for the two systems. System A may be regulated at the level of transcription[9,26] and System L at the level of translation[28].

In summary, the adaptive responses of transport Systems A and L described in this review are both mechanisms by which cells coordinate the uptake of amino acids with their nutritional requirements, allowing adaptation to changes in amino acid availability.

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Guided by electrostatics, a textbook protein comes of age

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The relationship between structure and function in the evolutionary-conservative cytochrome c family has remained fragmentary and confused despite a great deal of information, including more than 100 amino acid sequences and several crystal structures. Now, taking into account the long-ignored electrostatic properties of this versatile hemoprotein and those of its physiological reaction partners, a surprisingly simple and general model for their kinetics of interaction and for the corresponding electron-transfer complexes is emerging.

Following Keilin's rediscovery and establishment of the function of cytochrome in 1925, in his classical paper entitled 'A Respiratory Pigment, Common to Animals, Yeast and Higher Plants' (*Proc. Roy. Soc. Series B*, 98, 312–339), the one easily prepared, water-soluble component of this complicated electron transport system of the inner membrane of mitochondria, cytochrome *c*, has been studied more extensively than most other proteins[1,2]. Cytochrome *c* was the third protein to have its amino acid sequence determined and now that information is known for well over 100 species of eukaryotes and prokaryotes[3]. X-ray crystallographic structures are

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