REVIEW ARTICLE

Regulatory and molecular aspects of mammalian amino acid transport

John D. McGIVAN* and Marçal PASTOR-ANGLADA

*Department of Biochemistry, School of Medical Sciences, University Walk, Bristol BS8 1TD, U.K., and †Departament de Bioquimica i Fisologia, Universitat de Barcelona, Av. Diagonal 645, 08028 Barcelona, Spain

INTRODUCTION

The transport of amino acids across the plasma membranes of mammalian cells is a process of fundamental physiological importance and has been the object of extensive study. The characteristics, kinetics and regulation of various transport systems have been understood in general terms for many years, but until recently little was known about the structure and molecular properties of amino acid transport proteins. The great advances made in the cloning of genes for some of these transport proteins since 1991 have provided the impetus for the present review. The review will concentrate on the recent advances made in this area and also on a major outstanding problem in mammalian amino acid transport, i.e. the regulation of the major inducible amino acid transport system, known as System A. These topics to a large extent reflect the interests of the authors. Various aspects of amino acid transport are comprehensively covered in recent reviews [1,2] and in articles in a recently published book [3].

Initial work on the systematic characterization of amino acid transport systems was performed on Ehrlich ascites cells by Christensen and collaborators in the 1950s and 1960s (e.g. [4,5]) and similar investigations were subsequently performed by these and by other investigators to characterize amino acid transport in many other cell types, notably erythrocytes, hepatocytes and fibroblasts. The following general properties were established: first, amino acid transport systems in general are highly stereospecific, transporting L-amino acids much faster than D-amino acids. Secondly, these transport systems have a rather low substrate specificity when compared with most enzymes; thus a number of different amino acids may be transported on one transport system and in general a number of different transport systems with overlapping specificities may occur in the same cell membrane. Thirdly, transport systems can be divided into two categories, i.e. those that catalyse amino acid uniport and those that catalyse electrogenic Na^+–amino acid symport. In the second case the electrochemical potential gradient of Na^+ drives amino acid accumulation across the plasma membrane.

Kinetic and specificity studies on amino acid transport systems have been widely performed on intact cells and on membrane vesicles. Methods used in these studies have been reviewed elsewhere, as have the criteria for discriminating between different transport systems in the same membrane [6–8]. If two amino acids are transported on a common carrier, and this is the only major route of transport, then the transport of each amino acid should be competitively inhibited by the other and the K_i for inhibition of transport of one amino acid by another should equal the K_t value for the transport of the inhibitory amino acid [9]. The importance of establishing these criteria has not always been fully recognized, and in some early reports multiple transport systems were postulated where it is now apparent that fewer systems of broader specificity exist.

SPECIFICITY AND DISTRIBUTION OF TRANSPORT SYSTEMS

The major systems responsible for amino acid transport in mammalian cells, as determined by inhibition and kinetic studies, are summarized in Table 1. Most cells express the transport systems known as A, ASC and L. However, different cell types have different complements of additional transport systems which are required for their specific physiological function.

System A occurs in nearly all cell types. It catalyses the symport of most small aliphatic amino acids with Na^+ ions. Alanine, serine and glutamine are particularly good substrates. This system is characterized by the fact that it will also transport *N*-methylated amino acids [5], by tolerance of substitution of Li^+ for Na^+, and by the property of trans-inhibition. Transport activity is also highly pH-sensitive and decreases markedly with a decrease in pH. The non-metabolizable alanine analogue (*N*-methylamino)-α-isobutyric acid (methyl-AIB) is a substrate for this system. In many cases, competitive inhibition of uptake of a substrate by methyl-AIB is used to assess that proportion of substrate uptake which is mediated by System A. An important feature of System A is that in many cell types its activity is increased by a variety of factors, including amino acid starvation, hormones, growth factors and hyperosmotic stress. Mechanisms involved in the regulation of this transport system are considered in more detail later.

The specificity of the Na^+–amino acid symport System ASC overlaps with that of System A. Preferred substrates are alanine, serine and cysteine, but other aliphatic amino acids are also transported [10]. This system is relatively pH-insensitive, does not transport *N*-methylated substrates, and shows the property of trans-stimulation. System ASC is not inducible.

System L catalyses Na^+-independent transport of branched-chain and aromatic amino acids; aliphatic amino acids are relatively poor substrates. This system also catalyses amino acid antiport and may function physiologically in some circumstances to catalyse efflux rather than uptake [1]. The fact that branched-chain and aromatic amino acids are substrates for System L but are not in general substrates for Systems A and ASC means that these amino acids are not transported with Na^+ in most cell types. These compounds are relatively poorly metabolized and there is no metabolic requirement for their accumulation.

Basic and acidic amino acids are transported on systems which are distinct from those for neutral amino acids. System y^+ is a widely distributed system which catalyses the Na^+-independent transport of basic amino acids. This system is electrogenic and amino acid accumulation is driven by the cell plasma membrane potential. The acidic amino acids L-glutamate and L-aspartate

Abbreviations used: methyl-AIB, (*N*-methylamino)-α-isobutyric acid; EGF, epidermal growth factor; NEM, *N*-ethylmaleimide; ODC, ornithine decarboxylase; PDGF, platelet-derived growth factor; CEF, chick-embryo fibroblast; MDCK, Madin–Darby canine kidney; GABA, γ-aminobutyric acid; MuLV, murine leukaemic virus.

Table 1 Some major amino acid transport systems in mammalian cells

System	Specificity	Distribution	Comments
I Na^+-dependent transport systems			
A	Small aliphatic amino acids; methyl-AIB	Widespread	Inducible by starvation, hormones, growth factors
ASC	Small aliphatic amino acids; not methyl-AIB	Widespread	Not usually inducible
N	Glutamine, histidine, asparagine	Liver	Variant; System N^m in skeletal muscle
Gly	Glycine, sarcosine	Liver, erythrocytes, brain	Restricted distribution
B^0	Broad specificity; most neutral amino acids	Renal epithelial cells and brush-border membranes	Transports branched-chain and aromatic amino acids, unlike ASC
B	Broad specificity; most neutral amino acids	Intestinal epithelial cells	May be the same as System B^0
$B^{0,+}$	Broad specificity; most neutral acids and basic amino acids	Blastocysts, *Xenopus* oocytes, fibroblasts	Restricted distribution; variant of B and B^0?
X_{AG}^-	Glutamate, aspartate	Widespread	Electrogenic; more than one Na^+ per amino acid; high affinity
β	β-Alanine, taurine	Widespread	Also requires Cl^-
II Na^+-independent systems			
L	Mainly branched-chain and aromatic amino acids	Widespread	Not usually inducible
y^+	Lysine, histidine, arginine	Widespread	Electrogenic
$b^{0,+}$	Neutral and basic amino acids	As for System $B^{0,+}$	
x_c^-	Glutamate, cystine	Hepatocytes, fibroblasts	Electroneutral

are accumulated in many cells by a high-affinity transport system termed System X_{AG}^-; as the Na^+:glutamate stoichiometry is greater than 1:1, transport is again electrogenic. This system shows anomalous stereospecificity in that D-aspartate is also a substrate while D-glutamate is not [11]. A number of other glutamate transport systems with different kinetic properties and ion requirements occur in neural tissue [12].

Some additional transport systems are restricted to particular tissues. Following the observation that alanine and glutamine were transported on different systems in hepatocytes [13], a Na^+-dependent transport system (System N) for glutamine, histidine and asparagine was characterized in these cells [14]. So far the occurrence of this system has not been firmly established in other cell types, although a system with somewhat similar properties termed, System N^m, has been characterized in skeletal muscle [15]. System Gly, a transport system specific for glycine and sarcosine [16], occurs in some specific cell types, although glycine may be a substrate also for System A in the same cells. A transport system for β-alanine and taurine has also been characterized in some tissues [17]. The physiological significance of the occurrence of these systems is not yet clear.

Epithelial cells appear to possess a rather different complement of transport systems. A Na^+-dependent transport system of unusually broad specificity was characterized in rabbit intestinal [18] and pig jejunal [19] brush-border membrane vesicles. This system was originally termed System NBB, but has since been renamed System B in the interests of consistency with other broad-specificity transporters [20]. This transport system has been shown to occur in the lower intestine of many species and in the human colon carcinoma cell line Caco-2. A specific Na^+-dependent system for proline (System IMINO) occurs only in intestine.

In bovine renal brush-border membranes it was shown by a number of criteria that alanine, glutamine, leucine and phenylalanine were co-transported with Na^+ on a single common transport system, termed System B^0 to indicate a transport system of broad specificity which interacts only with amino acids having no net charge [21]. System A was absent from bovine renal brush-border membranes. The existence of this broad-specificity system can be rationalized physiologically as it allows the Na^+ gradient to drive the reabsorption of branched-chain and aromatic as well as aliphatic amino acids from the renal filtrate. Although the proteins corresponding to these transport systems have not yet been identified, it is probable that Systems B from intestine and B^0 from kidney are closely related. The bovine renal epithelial cell line NBL-1 also expresses a broad-specificity Na^+-dependent transport system with which glutamine, alanine, leucine and phenylalanine interact competitively [22]. Confluent cultures of NBL-1 cells do not express System A under normal conditions. There is some evidence that this may be the case also in confluent cultures of other renal epithelial cell lines (e.g. [23]).

A Na^+-dependent system of even broader specificity (System $B^{0,+}$) has been characterized in mouse blastocysts [24]. This transports both neutral and basic amino acids, and its expression is confined to a relatively small number of specific cell types. A Na^+-independent transporter of similar specificity ($b^{0,+}$) also occurs in some tissues. The occurrence of these two systems in *Xenopus* oocytes is a complicating factor in expression cloning of amino acid transporters. Again System $B^{0,+}$ may be expected to be related to the other broad-specificity systems described. The molecular mechanisms by which these transporters recognize such a wide range of substrates will be of particular interest, but their elucidation awaits cloning of the appropriate genes.

SYSTEM A: THE MAJOR REGULATED Na^+-DEPENDENT AMINO ACID TRANSPORT SYSTEM

As described above, the transport activity termed System A catalyses the Na^+-dependent accumulation of the small aliphatic amino acids which are metabolized most readily by cells as a source of energy and amino groups. The activity of this transport system in many different cell types is subject to modification by a wide range of factors, a number of which are of physiological

importance. Earlier work on the regulation of System A has been reviewed (e.g. [25–27]). Many aspects of this regulation, in particular the molecular mechanism of System A induction, are still unclear. The next section reviews accepted principles and recent developments in this area.

Hormonal stimulation of System A: characteristics and physiological relevance

Short-term regulation

Energetic aspects of Na^+–amino acid co-transport have been reviewed [28]. The stoichiometry of Na^+–amino acid symport via System A has been shown to be 1 : 1 in mouse ascites tumour cells [29], hepatocytes [30] and fibroblasts [31]. The process is thus electrogenic and is responsive to the membrane potential. A change in membrane potential could affect either the K_m or the $V_{max.}$ of the transport process or both, depending on the assumptions made about the exact molecular mechanism involved [32].

Glucagon [33] and epidermal growth factor (EGF) [34] stimulate alanine uptake into hepatocytes over a period of 30 min by a protein-synthesis-independent mechanism. It was suggested that this short-term stimulation was due to membrane hyperpolarization [33]. Effects of cyclic AMP on hepatocytes were studied in more detail in an investigation where $^{36}Cl^-$ distribution was used to determine the cell membrane potential [35]. Addition of cyclic AMP caused membrane hyperpolarization, which was accompanied by stimulation of alanine transport and also by an increase in Na^+,K^+-ATPase. The effects of cyclic AMP on transport could be mimicked by the ionophore nigericin which catalyses Na^+/H^+ exchange. From these and other considerations, it was concluded that glucagon and cyclic AMP exert their effect primarily by stimulation of the Na^+/H^+-exchange system in the hepatocyte membrane. This causes an increase in intracellular Na^+, consequent stimulation of the electrogenic Na^+,K^+-ATPase, membrane hyperpolarization and stimulation of Na^+–alanine symport. Activation of the Na^+/H^+ exchanger in hepatocytes by cyclic AMP and by EGF was later demonstrated directly [36]. It may be speculated that the liver isoform of the Na^+/H^+ exchanger contains a protein kinase A phosphorylation site. Such a site has been demonstrated in the isoform of the Na^+/H^+ exchanger (βNHE) cloned from trout [37], but not so far in the Na^+/H^+ exchanger cloned from other sources.

Hormonal regulation of amino acid transport via membrane hyperpolarization in liver is likely to be of physiological significance in the short-term control of gluconeogenesis. Measurement of intracellular and extracellular alanine concentrations during perifusion of hepatocytes demonstrated that plasma membrane transport of alanine is a major control step in alanine metabolism [38]. This conclusion is supported by other work [39,40].

Long-term regulation

System A is under hormonal and nutritional control in all cell types so far studied. Many physiological and pathophysiological situations characterized by an altered hormonal status result in significant alterations in the $V_{max.}$ of System A activity, which probably depends on the synthesis of new transporter molecules.

Insulin is perhaps the only hormone that up-regulates this transporter in a wide range of isolated cell types. In liver parenchymal cells the insulin effect is dependent on gene transcription, protein synthesis and glycosylation (see [25–27]). An interesting feature of insulin action is the evidence that most physiological situations associated with System A up-regulation in liver parenchymal cells are characterized either by insulin deficiency or by resistance to the hormone. Some examples are: diabetes [41–43] where skeletal muscle also shows increased System A activity [44], starvation [45–47], pregnancy [48,49], and post-natal development [50–52]. Insulin also down-regulates the liver System A activity of streptozotocin-induced diabetic rats, either when added to cells in culture or after administration *in vivo* [41,42]. On the other hand, insulin at high concentrations increased the activity of liver System A *in vivo* in experiments using the euglycaemic hyperinsulinaemic clamp technique [53]. The insulin effect was lower in fasted than in fed rats. The ability of insulin to induce System A in isolated liver parenchymal cells was also decreased in other situations, such as ethanol treatment of cultured hepatocytes [54], incubation of hepatocytes with amino acids (especially System A substrates) in concentrations corresponding to those found in the portal vein of rats fed a high-protein diet [55], in isolated hepatocytes from fetuses, newborn rats and rats that had undergone partial hepatectomy [51,56,57], and in cultured hepatocytes from adult and suckling rats fed a low-protein diet (M. Gómez-Angelats, B. Ruiz-Montasell, A. Felipe, F. J. Casado and M. Pastor-Anglada, unpublished work). In all these conditions the responsiveness of System A to glucagon was also impaired.

Glucagon also up-regulates System A activity in liver parenchymal cells when administed either *in vitro* [33,59] or *in vivo* [60–65]. The action of insulin and glucagon on liver System A activity is an exception to the classical view that these pancreatic hormones exert opposite effects on liver metabolism. The mechanism by which these hormones induce System A activity is still unclear; however, it appears that *in vivo* the role of glucagon may be more relevant.

Catecholamines up-regulate System A activity in liver parenchymal cells predominantly through α-receptors [65]. Adrenalin also induces System A activity in isolated rat hepatocytes from fetuses and from neonates [51]. The induction results, as in the adult, in a $V_{max.}$ effect which is blocked by both actinomycin D and cycloheximide. Conversely to the adult, the induction in fetuses and neonates is β-mediated and is not dependent on Ca^{2+}. Thus the sensitivity of System A to hormones seems to depend on the developmental stage of the animal.

Many other hormones and growth factors affect System A activity, although these effects may depend on multiple variables. For instance, glucocorticoids enhance System A activity by themselves in isolated rat hepatocytes, but not in long-term cultures, where they show only a synergistic effect with glucagon (see [27,66]).

These results reflect the complex regulation of System A *in vivo*, which depends on the interaction of different hormones and growth factors which are able to modulate its activity.

System A activity in proliferating normal cells and transformed cells

System A activity is dependent on cell density and on cell-cycle progression, and correlates with cellular growth in many cell types [67–73]. In general System A activity is also increased in chemically and virally transformed cells [67,71,73–76].

The dependence of System A activity on the cell cycle can be monitored *in vivo* when analysing the normal proliferative response of liver cells after partial hepatectomy. Induction of System A activity in regenerating rat livers was reported many years ago [77,78] and later recognized as a permissive step in the early phases of liver regeneration [79]. Although membrane hyperpolarization after partial hepatectomy presumably triggers

a short-term increase of System A activity, a stable induction was found in plasma membrane vesicles from regenerating rat livers [80,81] and was independent of the Na^+-transmembrane gradient [81]. Interestingly, the recently characterized Na^+-dependent nucleoside transporter(s) of liver parenchymal cells [82] is also up-regulated in the early phases of liver regeneration [83]. These increased transport activities contribute to the provision of amino acids and nucleosides which are essential substrates for cell growth, and are balanced by a co-ordinate increase in Na^+,K^+-ATPase expression [84].

In general, many physiological situations associated with liver hyperplasia such as pregnancy, lactation and fetal and neonatal development are associated also with an increase in the activities of Na^+-dependent amino acid transporters, especially System A [48,50,52,85,86]. These adaptations allow the liver to take up circulating amino acids much more efficiently [48,87–89].

After partial hepatectomy the up-regulated System A activity is inhibited by *N*-ethylmaleimide (NEM) with the same sensitivity as is the basal activity [81]. However, it has been reported that in most hepatoma cell lines System A activity is either insensitive or highly resistant to NEM treatment [90,91]. It is not yet known whether this is due to the expression of different isoforms having similar biological activity or to post-translational modification of the carrier proteins in tumour cells.

An unusual aspect of the role of amino acid transporters in the proliferative response of mammalian cells is the permissive effect of certain amino acids on the induction of a key enzyme involved in cellular growth processes, ornithine decarboxylase (ODC; EC 4.1.1.17). ODC catalyses the rate-limiting step in polyamine biosynthesis. Increased levels of this enzyme and of polyamines is a general property of proliferating tissues [92]. The stimulatory effect of insulin and growth factors such as EGF, nerve growth factor and platelet-derived growth factor (PDGF) on ODC activity is dependent on the intracellular levels of permissive amino acids [93,94]. These amino acids are substrates of Na^+-dependent transporters, especially Systems A and N. A non-metabolizable analogue, AIB, is also a potent inducing amino acid [93], suggesting that amino acid metabolism is not a requisite for ODC induction. The mechanisms for such regulation are not well understood. Asparagine, the major inducer, stimulates the synthesis of ODC at a translational step and can also, at least in part, suppress enzyme degradation [95].

Another regulatory link between ODC activity and amino acid transporters arises from recent evidence that System A substrates may induce cell volume swelling [96–99], which may also increase ODC activity [100]. Polyamines also contribute to the up-regulation of amino acid transport systems. In rat hepatocytes, the stimulatory effect of glucagon and cyclic AMP on AIB uptake is potentiated by spermidine, putrescine (the product of ODC activity) and cadaverine, by a mechanism which appears to involve the synthesis and effective insertion into the plasma membrane of new transporters [101]. In physiological conditions associated with liver cellular growth, such as regeneration [102] and pregnancy [103,104], significant induction of ODC activity occurs concomitantly with up-regulation of System A.

Regulation of System A by changes in osmolarity

System A activity is up-regulated after a hypertonic shock in chick-embryo fibroblasts (CEFs) [105], rat thymocytes [106], human fibroblasts [99,107], the renal epithelial cell line NBL-1 [108] and CHO-K1 cells (B. Ruiz-Montasell, M. Gómez-Angelats, F. J. Casado, A. Felipe and J. D. McGivan, unpublished work). When CEFs were grown in a high-NaCl medium (200 mM instead of the usual 143 mM Na^+ concentration), a selective induction of methyl-AIB-inhibitable L-proline uptake (System A) occurred, while System ASC and System L activities remained unaltered [105]. System A induction was blocked by cycloheximide and actinomycin D. Thus induction of System A activity after hypertonic shock requires *de novo* synthesis of new carriers and/or other related regulatory proteins. Exposure of cells to high Na^+ concentrations resulted in a rapid increase in intracellular Na^+ levels and a 50% decrease in L-leucine incorporation into proteins, suggesting that protein synthesis was partially impaired [110]. Several considerations rule out the possibility that these effects of hypertonic medium are mediated by either an increased inward Na^+ electrochemical gradient or increased intracellular Na^+ levels: (a) System A activity was induced in fibroblasts, thymocytes, NBL-1 and CHO-K1 cells no matter what agent was used to increase medium osmolarity (KCl, NaCl, sucrose, choline chloride, *N*-methylglucamine or mannitol) ([106,108,110]; B. Ruiz-Montasell, M. Gómez-Angelats, F. J. Casado, A. Felipe and J. D. McGivan, unpublished work); (b) in rat thymocytes, hypertonic shock induced a slight increase in the intracellular Na^+ concentration (from 33 to 37 mM) and a decrease in membrane potential (from -56 to -50 mV); thus the Na^+ electrochemical gradient was in fact decreased after exposure of cells to hypertonic medium [106]; (c) the decreased rates of protein synthesis observed in hypertonic medium were similar to those in cells cultured in isotonic medium where NaCl had been replaced by choline chloride or by other osmolytes [111,112]; (d) up-regulation of System A in CEFs cultured in isotonic medium occurred when NaCl was replaced by impermeant disaccharides such as sucrose, but not by readily permeant monosaccharides such as glucose [112]. However, both media induced low protein synthesis rates and low intracellular Na^+ concentrations.

The molecular mechanisms involved in System A up-regulation after hypertonic shock have not yet been elucidated. It has been suggested that cell shrinking is an important factor in triggering System A induction after exposure of cells to hyperosmotic medium. Cell shrinking occurred when CEFs were cultured in isotonic medium supplemented with sucrose, but not with glucose [112]. NBL-1 cells also shrink rapidly after hypertonic shock [108]. The time course of the resultant System A induction was in the range of that described for System A gene derepression (adaptive control). Induction of System A activity in NBL-1 cells cultured in 200 mM sucrose occurred only after System A activity had emerged after previously culturing the cells for 12 h in amino-acid-free medium; otherwise, no effect induced by hypertonicity was observed [108]. Interestingly, in the alanine-resistant mutant CHO-K1 *ala*^{r}4, which has an impaired ability to respond to adaptive control, System A activity was induced by hypertonic medium to values similar to those described in the wild type, CHO-K1 (B. Ruiz-Montasell, M. Gómez-Angelats, F. J. Casado, A. Felipe and J. D. McGivan, unpublished work). This strongly supports the view that these processes occur via independent mechanisms. Furthermore, while hypertonicity-induced up-regulation of System A was sensitive to the microtubule-disrupting agent colcemid in starved NBL-1 cells, colcemid did not block the adaptive response [108]. In both NBL-1 and CHO-K1 cells the adaptive up-regulation of System A by amino acid starvation was blocked by the glycosylation inhibitor tunicamycin, but the effect of hyperosmotic medium was not (B. Ruiz-Montasell, M. Gómez-Angelats, F. J. Casado, A. Felipe and J. D. McGivan, unpublished work). These results suggest that exposure to hyperosmotic medium may result in the synthesis of one or more proteins which activate System A transporters rather than increased synthesis of the System A transport protein itself.

Attempts to identify hyperosmolarity-induced stress proteins, perhaps related to System A up-regulation, were reported several years ago [110,111,113]. It was shown that, during the period of cell recovery after returning the cells to isotonic medium, some relatively low-molecular-mass proteins of less than 100 kDa were induced and other large polypeptides of more than 140 kDa were significantly repressed [113]. None of the induced major bands corresponded to the recently suggested molecular mass of the System A protein(s).

There is increasing evidence that some plasma membrane ion transporters may be structurally and functionally linked to the cytoskeleton. The Na^+,K^+-ATPase may form complexes with ankyrin and fodrin in Madin–Darby canine kidney (MDCK) cells [114,115]. Ankyrin has also distinct domains that allow for *in vitro*-selective and specific binding to Na^+,K^+-ATPase and the anion exchanger (Band 3) [116]. In the toad kidney A6 cell line, treatments leading to different levels of polymerization of the actin filaments indicated a key role for the actin network in the epithelial Na^+-channel activity [117]. In the human intestinal epithelial cell line T84, cyclic AMP-elicited Cl^- secretion was dependent on remodelling of F-actin filaments and was blocked by the stabilization of F-actin after binding to nitrobenzoxadiazole–phallicidin [118]. Cl^- secretion seems to be mainly mediated by activation of the $Na^+/K^+/2Cl^-$ co-transporter. [119]. Direct evidence for binding of the carrier to cytoskeleton components is not, as yet, available. Similarly, specific regions involved in the opening of CIC-2 Cl^- channels by cell volume have been identified in *Xenopus* oocytes [120], and PC12 cells express an ion-channel activity the gating of which depends on the medium osmolarity and on the integrity of the microfilament, but not the microtubular network [121]. That there may be a regulatory link between cytoskeletal proteins and amino acid transporters is an interesting possibility, but no direct evidence is at present available on this point.

The physiological significance of the adaptation of amino acid transport to hyperosmotic stress is not altogether clear. A likely possibility is that amino acids may act as osmolytes, at least in renal epithelial cells. MDCK cells release a substantial amount of intracellular amino acids when cultured in hypotonic medium [122,123], depleting the free amino acid pool from 69 to 25 mM [124]. However, amino acid efflux seems to be accounted for, at least partially, by anion channels [125] and involves rapid effects which do not depend on the synthesis of new carrier proteins. Similarly, induction of hepatic System N by hypotonicity or cell swelling triggered by Na^+-coupled uptake of amino acids is cycloheximide-insensitive and does not involve either trans-stimulation or recruitment of additional carriers [126].

Two possibly analogous examples of induction of Na^+-dependent osmolyte transport by hyperosmotic medium have been reported recently. Betaine and *myo*-inositol behave as osmolytes in MDCK cells. As described later, Na^+-dependent transporters for betaine (BGT1) [127,128] and for *myo*-inositol (pSMIT) [129,130] have been cloned from this cell line. The levels of mRNA coding for BGT1 and for pSMIT markedly increase soon after hypertonic shock. It has been demonstrated recently by run-on assays that exposure of MDCK cells to hypertonic medium enhances the transcription rate of the betaine transporter [131]. These results indicate that anisotonicity is able to regulate gene expression, as already reported for the transcription of genes coding for microfilament proteins [132,133].

Models of System A regulation

Several models for the long-term regulation of System A activity have been proposed during the past decade [60,134,135]. Each model comes from data on a different cell type, which might explain some of the discrepancies between them. It may be assumed that although System A activity is ubiquitous, there might be isoforms that are differentially regulated and expressed in different cells.

Kilberg and co-workers [26,60,136] analysed the substrate-dependent repression of System A activity previously induced by hormone treatment or by experimental diabetes. Primary cultures of hepatocytes from rats that had been injected with glucagon 4–6 h before hepatocyte isolation displayed high System A activities when cultured in an amino-acid-free medium. When cells were exposed to an amino-acid-supplemented medium, a decay of stimulated System A activity occurred progressively, indicating a short half-life for the putative System A protein(s) (1.5–2.5 h). This effect was mimicked by individual amino acids, generally System A-preferring substrates [137], and was blocked by actinomycin and cycloheximide [26,60,136,137]. These results formed the basis of a model which originally postulated that amino acids could enhance the transcription of a gene coding for a protein which inactivated System A activity, that is the so-called **T**ransport **I**nactivating **P**rotein, TIP [26,60]. Later evidence suggested that TIP is a non-glycosylated protein and is coded by an mRNA lacking a poly(A) tail, consistent with the idea that the putative TIP is a nuclear regulatory protein, probably a histone [136,137], acting on the System A gene(s), rather than a direct inactivator of the transport protein(s). This model indirectly assumed that changes in free amino acid levels would be detected by an amino-acid-binding molecule.

The model by Englesberg and co-workers (Figure 1a) [134,135,138] is based on extensive work using somatic cell genetics. Again a regulatory protein (R1) is postulated to control System A gene expression. CHO-K1 cells express the major transport systems involved in neutral amino acid transport, i.e. Systems A, ASC and L [139,140]. Adaptive control of System A in CHO-K1 cells is also inhibited by cycloheximide, actinomycin D and selected amino acids, although not necessarily System A substrates [134]. The best evidence in favour of a repressor gene comes from the study of a single mutant, CHO-K1 *ala*^{r}4, which has basal transport rates through System A similar to those of the derepressed wild type and has lost the ability to respond further to amino acid starvation [134]. Assuming that a single mutational event is responsible for the CHO-K1 *ala*^{r}4 phenotype, and taking account of the evidence that somatic cell hybrids CHO-K1 × CHO-K1 *ala*^{r}4 show the wild-type phenotype, it was postulated that the mutation involved a regulatory protein instead of the carrier itself. The work on CHO cells [134,141] and that on rat hepatocytes [26] showed that those amino acids able to block the adaptive response are not necessarily System A substrates; this is consistent with the involvement in the induction of System A activity of another protein having different amino-acid-binding specificity. The mechanisms of System A induction in the two cell types may well be different, i.e. the decay of induced activity after re-addition of amino acids is dependent on protein synthesis and gene transcription in rat hepatocytes but not in CHO cells or in NBL-1 cells.

Induction of System A activity by amino acid starvation and incubation in hypertonic medium in the NBL-1 cell line was described in the previous section. These results are consistent with a further model in which the proteins induced by starvation and by hypertonic medium are different (Figure 1b).

At present, the lack of molecular probes or appropriate System A antibodies has prevented the assessment of any of these models and they should be considered as working hypotheses, although all are supported by some experimental evidence.

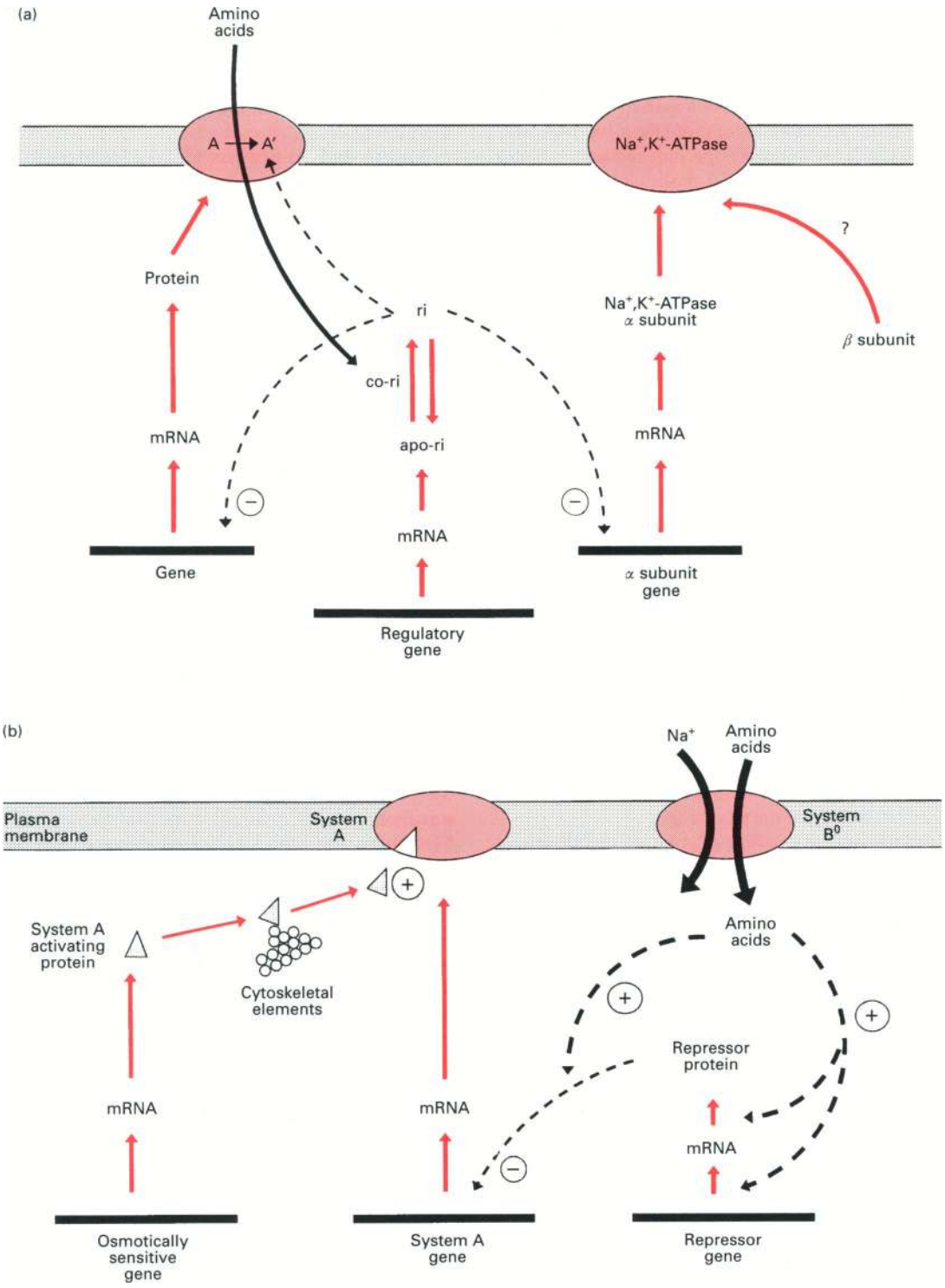

Figure 1 Models for the regulation of System A

(**a**) Model for the co-ordinate regulation of System A and α-subunit Na^+,K^+-ATPase gene expression in CHO-K1 cells. The regulatory gene codes for an inactive repressor (apo-ri) that can be activated in the presence of amino acids (co-ri). The active form of the regulatory protein is at the same time a repressor of System A gene expression and an inactivator of the System A protein. ri can block expression of the gene coding for the α-subunit of the Na^+,K^+-ATPase. See the text for further details. Adapted from [161]. (**b**) Model for the regulation of System A activity in NBL-1 cells. Amino acids completely block System A expression either by acting on a putative repressor protein or by enhancing the expression of the repressor itself. In the absence of amino acids System A is derepressed. An osmotically sensitive gene codes for a putative System A-activating protein. This protein may interact with cytoskeletal elements to exert its function or may itself be a component of the cytoskeleton. In the presence of amino acids System A is not expressed and the activating protein cannot up-regulate activity. The data on which this model is based are taken from [108].

Induction of System A in relation to other Na^+-coupled transport systems

Many examples of simultaneous induction of Na^+-dependent amino acid transporters come from *in vivo* observations. Feeding a high-protein diet induced a co-ordinate induction of several Na^+-dependent amino acid transport systems (A, N, Gly, X_{AG}^-) [142]. Pregnant rats also have up-regulated systems A and ASC in liver [48], while in a model of genetic obesity, the Zucker rat, Systems A, ASC and N are up-regulated in liver plasma membrane vesicles [143]. All these models are characterized by tissue hypertrophia and/or hyperplasia, and there is no direct evidence for a common pathway mediating the co-ordinate induction of Na^+-dependent transporters. However, alanine-resistant CHO-K1 mutants show pleiotropic phenotypes leading to an increase in the activities of Systems A, ASC and a so-called System P [138].

Induction of Na^+-coupled concentrative transporters should be sustained by an increased ability to regenerate the Na^+ transmembrane gradient. Indeed, in some cell types the concentrative uptake of amino acids is a major determinant of Na^+,K^+-ATPase activity [144–148]. Several physiological models show apparent co-ordinate changes of Na^+,K^+-ATPase and Na^+-dependent amino acid transport activities. This is the case in livers from rats fed a high-protein diet [149], livers from rats early after partial hepatectomy [81,84,150], during post-natal development [50], in the obese Zucker rat (B. Ruiz-Montasell, A. Ferrer-Martínez, A. Felipe, J. Casado and M. Pastor-Anglada, unpublished work) and during reticulocyte maturation [152]. Co-ordinate changes in these two activities were described many years ago in studies of cell-cycle progression in Ehrlich ascites tumour cells [153] and cell differentiation in Friend erythroleukaemia cells [154]. CHO cells when cultured for 24 h in a low-K^+ medium also showed a co-ordinate increase of Na^+,K^+-ATPase and AIB transport activities [155]. At that time the possibility that the two transporters were somehow genetically linked was considered. Prolonged ouabain treatment resulted in high expression of Na^+,K^+-ATPase in HeLa cells [156] and in increased activity in fibroblasts [76,157,158]. In both cases, a stable increase of System A activity was also observed.

More direct evidence of co-ordinate up-regulation of both transport activities comes from somatic cell genetics, using CHO-K1 cells and the alanine-resistant mutants described above. A close relationship between the $V_{max.}$ of System A and Na^+,K^+-ATPase activity was found when a whole set of independent mutants and revertants was analysed [159]. In particular CHO-K1 *ala*^{r}4, the CHO mutant unable to respond to adaptive control, showed a significant increase in Na^+,K^+-ATPase activity over the wild type, with an increase in the number of ouabain-binding sites and also in the amount of mRNA coding for the α_1 subunit [160]. Again, assuming that single mutational events are responsible for the phenotype of clones selected by alanine resistance, it was postulated that the two transporters were under co-ordinate control at the gene level (see Figure 1a). Further evidence was provided when analysing the adaptive response itself [161]. It was shown that the amount of α_1 message was increased after amino acid starvation in CHO-K1 cells, but the increase was blocked by single amino acids with a similar specificity to that described for System A up-regulation after amino acid deprivation. This interesting finding, along with the evidence that cell hybrids *WT–ala*^{r}4 showed the wild-type phenotype, is consistent with a regulatory gene linking Na^+,K^+-ATPase and System A gene expression (Figure 1a). Although much of the molecular biology of the Na^+,K^+-ATPase has already been elucidated, the lack of information on the molecular characteristics of System A and other amino acid transporters has so far prevented a direct assessment of the hypothesis of co-ordinate regulation.

IDENTIFICATION AND CHARACTERIZATION OF AMINO ACID TRANSPORT PROTEINS

Progress in the identification of mammalian amino acid transport systems has been considerably slower than that for bacterial transport systems or for other mammalian transport systems such as that for glucose. The major reason for this has been the lack of a suitable specific inhibitor which could be used as high-affinity labelling reagent in the way that cytochalasin B, for example, has been used as a label for the erythrocyte glucose transporter. Analogues of amino acids in general act as weak competitive inhibitors and are inappropriate for this purpose. Thiol reagents inhibit Na^+-dependent amino acid transport, but these reagents are unspecific. A further difficulty in approaches using labelling is the likely low abundance of amino acid transport proteins in cell membranes. While direct approaches to identification of transport have therefore proved difficult, there has been a great explosion of information based on the technique of expression cloning in the past two years. These developments will be reviewed in the following section.

Approaches involving direct identification of transport proteins

A number of reports describe the solubilization and reconstitution of amino acid transport activity into artificial phospholipid membranes. These include reconstitution of amino acid transport from pig cortex membranes [162], System A [163,164] and System N [165] from rat liver, and the broad-specificity system from bovine kidney [166]. In principle, once a reproducible reconstitution method has been established, the solubilized membrane proteins can be fractionated and progressively purer fractions containing transport activity can be isolated. Eventually this may lead to direct purification of the transport protein.

This approach led to the purification to homogeneity of the ($Na^+ + Cl^-$)-dependent γ-aminobutyric acid (GABA) transport protein from rat brain [167]. Peptides derived from this protein sequence were used to design oligonucleotide probes to screen a rat brain cDNA library [168]. The amino acid sequence derived from the cDNA clone encoding the transport protein (GAT-1) predicted a protein with 12 transmembrane helices. Injection of mRNA for this protein into *Xenopus* oocytes induced high-affinity Na^+- and Cl^--dependent GABA transport with characteristics similar to those of GABA transport in brain preparations.

A similar procedure was used to purify a Na^+/K^+-coupled glutamate transport protein of glial origin [169]. A rat brain cDNA expression library was screened with an antibody to this protein and a full-length clone (GLT-1) was derived [170]. Expression in HeLa cells induced glutamate transport with the expected properties. The predicted protein contained eight putative transmembrane helices. A second high-affinity glutamate/asparate transporter termed GLAST was cloned by screening a rat brain cDNA library with an oligonucleotide corresponding to a peptide derived from a purified brain glycoprotein [171]. GLAST mRNA was again confined to brain; the cDNA sequence predicted a 543-amino-acid protein with multiple transmembrane helices. Expression of GLAST in oocytes induced a high-affinity Na^+-dependent L-glutamate uptake with a likely stoichiometry of 3 Na^+:1 glutamate:1 K^+ [172]. In a similar approach, a 100 kDa protein from pig brain was purified to homogeneity and was demonstrated by reconstitution to be a ($Na^+ + Cl^-$)-dependent glycine transporter [173,174].

Attempts to isolate other transport proteins, particularly those for neutral amino acids, by this method have been less successful. Using reconstitution as an assay, a protein fraction with System A activity was isolated from Ehrlich ascites cells. An antibody to the major component immunoprecipitated transport activity from solubilized membranes [175]. No details of the protein with which this antibody reacts have yet been published. More recently, a monoclonal antibody was obtained which both inhibited System N activity in hepatocytes and immunoprecipitated transport activity from solubilized membranes [176]. Details of the sequence of this putative System N protein are awaited.

The Na^+-dependent broad-specificity transport system (System B^0) from bovine kidney brush-border membranes has been reconstituted into liposomes [166]. Monoclonal antibodies were made to the active protein fraction. One antibody immunoprecipitated reconstitutable transport activity from solubilized membranes. Further investigation [177] showed that this antibody specifically reacted with the brush-border membrane protein aminopeptidase N, an ectoenzyme with a single transmembrane helix. Aminopeptidase N forms extensive arrays in these membranes [178] which could therefore represent complexes with effective multiple transmembrane helices, but there is no evidence that this enzyme can actually act as a transporter. Removal of aminopeptidase N from the membranes using limiting concentrations of papain greatly increased the K_m values for Na^+-dependent amino acid transport without affecting the $V_{max.}$. Na^+-dependent glucose transport was unaffected. There is a close correspondence between the amino acids which are most readily hydrolysed from the N-terminal end of peptides by aminopeptidase N and those amino acids which are most readily transported. It was suggested [177] that the amino-acid-binding sites of aminopeptidase N and the transporter may interact in some way, affecting the kinetics of the overall transport process.

Expression cloning of amino acid transport proteins

In cases where the transport protein cannot be identified directly, the technique of expression cloning has been employed to isolate cDNA clones encoding the transporter. The first mammalian metabolite transporter to be cloned in this way was the intestinal Na^+-dependent glucose transporter in 1987 [179]. A number of subsequent reports describing expression of various amino acid transport activities on injection of total tissue mRNA into oocytes have appeared [180–187], but only in the last two years has this led to the isolation of clones for putative transport proteins.

The first mammalian amino acid transport system to be identified was System y^+ (now called cationic amino acid transporter 1 or CAT1), which was found to be identical with the mouse ecotropic virus receptor [188,189]. mRNA coding for this receptor was injected into *Xenopus* oocytes and uptake of amino acids was followed using radioactive tracers [188,189] or voltage-clamping [189]. Oocytes expressing the virus receptor catalysed the Na^+-independent electrogenic uptake of lysine, arginine and ornithine with K_m values similar to those for System y^+. Further, the uptake of basic amino acids was inhibited by homoserine, but only in the presence of Na^+; this is again consistent with the properties of System y^+. The tissue distribution of mRNA was also consistent with that of System y^+ activity. The mouse ecotropic virus receptor (EcoR) had been cloned previously [190]. This protein has 12–14 membrane-spanning domains and some structural similarity to the arginine and histidine permeases of *Saccharomyces cerevisiae*. Chronic infection of EcoR-transfected fibroblasts by ecotropic murine leukaemia virus (MuLV) resulted in down-regulation of System y^+ activity [191]. It appears that the transporter provides a binding site for an envelope viral protein, gp70, subsequently allowing infection [191,192]. Two isoforms of the transporter have so far been identified. CAT-1 codes for a high-affinity y^+ transport activity and is absent from liver. CAT-2, when expressed in oocytes, induces a low-affinity cationic amino acid transport activity. The CAT-2 protein is present in liver but does not confer susceptibility to amphotropic MuLV infection, at least in CHO cells [193,194].

At the same time as the GLAST and GLT-1 brain glutamate transporters were cloned (see above), a high-affinity glutamate/aspartate transporter (EAAC1) was identified by expression cloning from rabbit small intestine [195]. mRNA for this transporter was found also in kidney and brain. The kinetics and specificity of amino acid transport by this protein when expressed in *Xenopus* oocytes were similar to those of the transport system termed X_{AG}^- which is widely distributed. The three glutamate transporters EAAC1, GLT-1 and GLAST show marked similarities and represent a protein family (see [196,197]).

An extension of this approach in the search for amino acid transport proteins was to look for proteins related to the transporters whose identity had already been firmly established (the glutamate family, the GABA transporter and the Na^+-dependent glucose transporter). After the expression cloning of a neural ($Na^+ + Cl^-$)-dependent noradrenaline transporter [198] which was closely related to the GABA transporter, cDNA clones for neural ($Na^+ + Cl^-$)-dependent transporters for dopamine [199–201], 5-hydroxytryptamine [202], proline [203] and glycine [204–206] were isolated by screening libraries with oligonucleotides derived from the sequence of the GABA and/or the noradrenaline transporter. A ($Na^+ + Cl^-$)-dependent transporter for betaine was identified in MDCK cells by expression cloning [128]. Using probes to this sequence, a ($Na^+ + Cl^-$)-dependent transport system for taurine [207] was cloned in the same cell line. Another ($Na^+ + Cl^-$)-dependent transporter was similarly cloned from mouse brain [208]. All the members of this group of ($Na^+ + Cl^-$)-dependent neurotransmitter transporters show extensive sequence similarity to the GABA transporter and form a superfamily of proteins each with 10–12 putative transmembrane regions [209,210].

The Na^+-dependent neutral amino acid transport proteins continue to be elusive. By low-stringency screening of a cDNA library derived from LLC-PK1 cells with probes for the Na^+–glucose transporter, Kong et al. [211] obtained a cDNA clone (SAAT1) which, when used to transfect COS cells, induced Na^+-dependent methyl-AIB transport. It was suggested that the SAAT1 clone may be related to System A. If this is in fact the case, it would be of great interest and would open the way to a detailed study of the regulation of this transport system. The level of expression of this system in COS cells was, however, relatively low (only 2-fold over the control). Further evidence relating to the identification of SAAT1 with the System A transporter will presumably come from expression of the cDNA in oocytes, using electrophysiological techniques to detect electrogenic Na^+–amino-acid co-transport, as has been done for the EAAC1 glutamate carrier and for the CAT-1 System y^+ transporter. If SAAT1 does in fact encode System A, it should also be possible to show increased levels of SAAT-1-hybridizing transcripts on induction of System A activity by amino acid deprivation in a suitable cell type. Until such reports appear, it remains unclear whether the SAAT1 clone is truly related to the inducible System A transport activity.

Two recent publications report cloning and sequencing of another Na^+-dependent neutral amino acid transport system.

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ASCT1          MEKSNETNGYLDSAQAGPAAGPGAPGTAAGRARRCARFLRRQALVLL--TVSGVLAGA
EAAC1                                    MGKPAPKGCDSKRFLKNNWLLLS-TVVAVVLGI
GLT-1          MASTEGANNMPKQVEVRMHDSHLSSEEPKHRNLGMRMCDKLGKNLLLSLTVFGVILGA
GLAST     MTKSNGEEPRMGSRMERFQQGVRKRTLLAKKKVQNITKEDVKSYLFRNAFVLL--TVSAVIVGT

ASCT1     GLGAALR-GLSLSRTQVTYLAFPGEMLLRMLRMIILPLVVCSLVSGAASLDASCLGRLGGIRVA
EAAC1     VIGVLVREYSNLSTLDKFYFAFPGEILMRMLKLVILPLIVSSMITGVAALDSNVSGKIGL-RAV
GLT-1     VCGGLLRLAAPIHPDVVMLIAFPGDILMRMLKMLILPLIISSLITGLSGLDAKASGRLGT-RAM
GLAST     ILGFALRPY-KMSYREVKYFSFPGELLMRMLQMLVLPLIISSLVTGMAALDSKASGKMGM-RAV

ASCT1     YFGL-TTLSASALAVALAFIIKPGSGAQTLQSSDLGLEDSGPPPVPKETVDSFLDLARNLFPSN
EAAC1     LYYFCTTIIAVILGIVLVVSIKPGVTQKVDEIDRTGSTPEVST------VDAMLDLIRNMFPEN
GLT-1     VYYMSTTIIAAVLGVILVLAIHPGNPKLKKQLGPGKKNDEVSS------LDAFLDLIRNLFPEN
GLAST     VYYMTTTIIAVVIGIIIVIIIHPGKGT-KENMYREGKIVQVTA------ADAFLDLIRNMFPPN

ASCT1     LVVAAFRTYATDYKVV--------TQNSSSGNVTHEKIPIGTEI--------------EGMNIL
EAAC1     LVQACFQQYKTTREEV--TASDDTGKNGTEESVTAVMTTAVSENRTKEYRVVGLYS--DGINVL
GLT-1     LVQACFQQIQTVTKKVLVAPPS-EEANTTKAVISLLNETMNEAPEETKIVIKKGLEFKDGMNVL
GLAST     LVEACFKQFKTSYEKRSFKVPIQANETLLGAVINNVSEAMETLTRIREEMVPVPGSVN-GVNAL

ASCT1     GLVLFALVLGVALKKLGSEGEDLIRFFNSLNEATMVLVSWIMWYVPVGIMFLVGSKIVEMKDII
EAAC1     GLIVFCLVFGLVIGKMGEKGQILVDFFNALSDATMKIVQIIMCYMPLGILFLIAGKIIEVEDWE
GLT-1     GLIGFFIAFGIAMGKMGEQAKLMVEFFNILNEIVMKLVIMIMWYSPLGIACLICGKIIAIKDLE
GLAST     GLVVFSMCFGFVIGNMKEQGQALREFFDSLNEAIMRLVAVIMWYAPLGILFLIAGKILEMEDMG

ASCT1     VLVTSLGKYIFASILGHVIHGGIVLPLIYFVFTRKNPFRFLLGLLAPFATAFATCSSSATLPSM
EAAC1     IF-RKLGLYMVTVLSGLAIHSIVILPLIYFIVVRKNPFRFAMGMTQALLTALMISSSSATLPVT
GLT-1     VVARQLGMYMITVIVGLIIHGGIFLPLIYFVVTRKNPFSFFAGIFQAWITALGTASSAGTLPVT
GLAST     VIGGQLAMYTVTVIVGLLIHAVIVLPLLYFLVTRKNPWVFIGGLLQALITALGTSSSSATLPIT

ASCT1     MKCIEENNGVDKRISRFILPIGATVNMDGAAIFQCVAAVFIAQLNNIELNAGQIFTILVTATAS
EAAC1     FRCAEEKNRVDKRITRFVLPVGATINMDGTALYEAVAAVFIAQLNDMDLSIGQIITISVTATAA
GLT-1     FRCLEDNL-IDKRVTRFVLPVGATINMDGTALYEAVAAIFIAQMNGVILDGGQIVTVSLTATLA
GLAST     FKCLEENNGVDKRITRFVLPVGATINMDGTALYEALAAIFIAQVNNFDLNFGQIITISITATAA

ASCT1     SVGAAGVPAGGVLTIAIILEAIGLPTHDLPLILAVDWIVDRTTTVVNVEGDALGAGILHHLNQK
EAAC1     SIGAAGVPQAGLVTMVIVLSAVGLPAEDVTLIIAVDWLLDRFRTVVNVLGDAFGTGIVEKLSKK
GLT-1     SIGAASIPSAGLVTMLLILTAVGLPTEDISLLVAVDWLLDRMRTSVNVVGDSFGAGIVYHLSKS
GLAST     SIGAAGIPQAGLVTMVIVLTSVGLPTDDITLIIAVDWFLDRLRTTTNVLGDSLGAGIVEHLSRH

ASCT1     ATKKGEQELAEVKVEAIPNCKSEEETSPLVTHQNPAGPVASAPELESKESVL  532
EAAC1     ELEQMDVSSEVNIVNPFALESATLDNEDSDTKKSYINGGFAVDKSDTISFTQTSQF  524
GLT-1     ELDTIDSQHRMHEDIEMTKTQSVYDDTKNHRESNSNQCVYAAHNSVVIDECKVTLAANGKSADC
GLAST     ELKNRDVEMGNSVIEENEMKKPYQLIAQDNEPEKPVADSETKM  543

GLT-1     SVEEEPWKREK  573
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Figure 2 Similarities of amino acid sequences of the glutamate transporters and the Na^+-dependent neutral amino acid transporter ASCT1

The deduced amino acid sequences of ASCT1 [213] and the glutamate transporters EAAC1 [195], GLAST [171] and GLT-1 [170] are aligned. Identical amino acids are highlighted. The diagram is a modification of that in [213], but includes the updated sequence information for GLT-1 given in [196].

Table 2 Some cloned amino acid transporters

Clone	Tissue distribution	Corresponding transport system	No. of amino acids	Putative membrane helices	Reference
EcoR receptor (CAT family)	Widespread	y^+	622	14	192
EAAC1	Intestine, kidney, brain	X_{AG}^-	524	10	195
GLAST	Brain	Glutamate transporter	543	> 6	171
GLT-1	Brain	Glutamate transporter	573	8–9	170
GAT-1	Brain	GABA transporter	599	12	168
GLYT-1	Brain	'System Gly-like'	633	12	206
TAU	Widespread	System β?	655	12	207
TAUT	Brain	β-like	590	12	208
SAAT1	Widespread	System A?	660	11 as for SGLT1	211
SATT	Widespread	System ASC?	529	6–10	212
ASCT1	Widespread	System ASC?	532	Up to 10	213

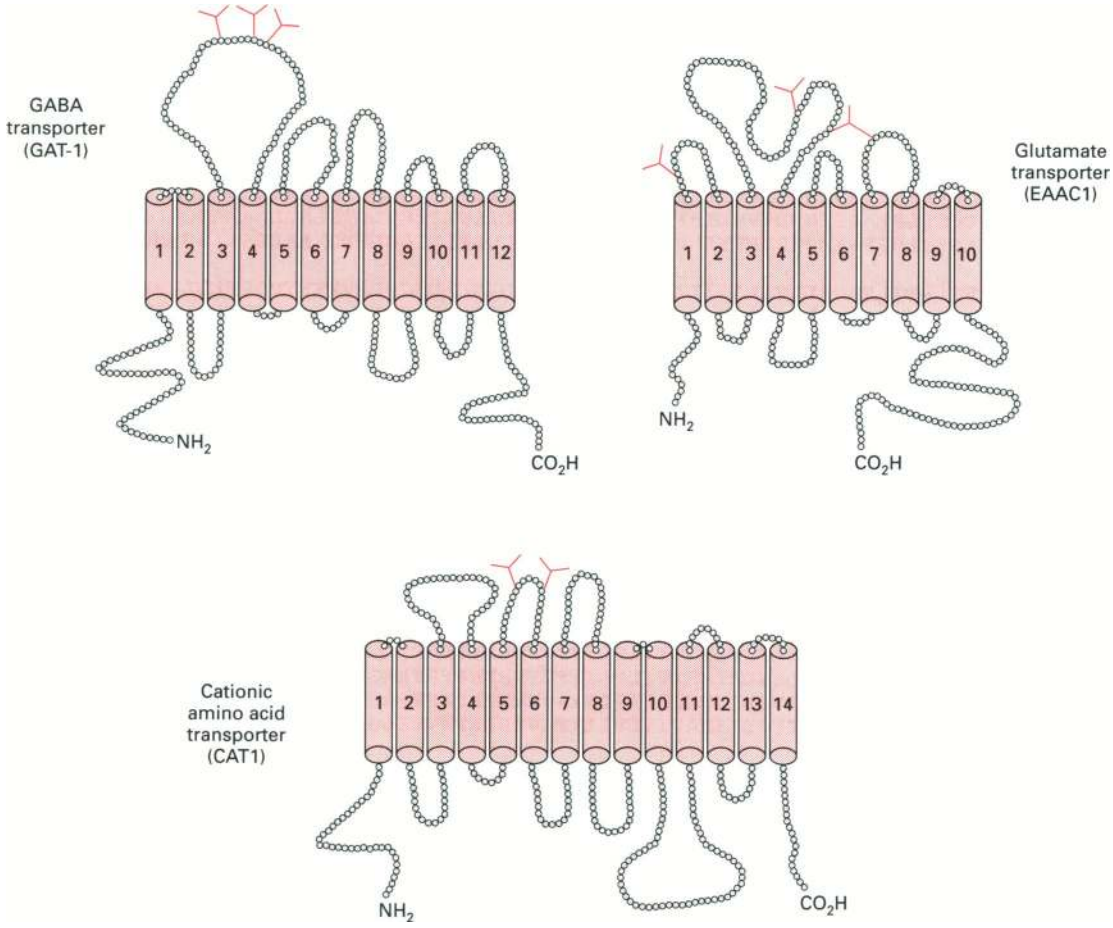

Figure 3 Amino acid transporters

The putative transmembrane orientations of the GABA transporter (GAT-1), the glutamate transporter (EAAC1) and the cationic amino acid transporter (CAT1) are shown as representatives of the three families of amino acid transporters cloned to date. Potential glycosylation sites on the cytoplasmic-facing side of the proteins are indicated (red). The structures are redrawn from the illustrations in references [168], [195] and [192] respectively.

Shafqat et al. [212] used PCR to obtain a cDNA probe from human hippocampus which had distant homology to an *Escherichia coli* glutamate/aspartate transporter. This probe was used to screen a human hippocampal cDNA library, and a full-length clone (SATT) was obtained which had 40% sequence similarity with mammalian glutamate transporters. Expression of this cDNA in HeLa cells induced low levels (70% increase over the control) of Na^+-dependent uptake of amino acids which were methyl-AIB-insensitive. It was proposed that this clone might be related to System ASC, but some anomalies were found. In particular, uptake of cysteine was not induced; this amino acid is a good substrate for System ASC in many cells.

At the same time, Arriza et al. [213] reported isolation of another cDNA clone by screening a library with oligonucleotide probes derived from the sequence of the glutamate transporter GLAST. When this clone (ASCT1) was expressed in *Xenopus* oocytes, electrogenic Na^+-dependent uptake of alanine, serine and cysteine among other amino acids was observed by the sensitive voltage-clamping technique. It was concluded that clone ASCT1 probably represented System ASC. The amino acid sequences encoded by the two clones ASCT1 and SATT are in fact almost identical (93% identity). Again the exact biochemical functions of the proteins encoded by these clones await confirmation. The similarities between the primary structures of ASCT1 and the three glutamate transporters GLAST, GLT-1 and EAAC1 are illustrated in Figure 2 [213].

Table 2 summarizes some details of the amino acid transport proteins identified so far. The postulated transmembrane orientation of transporters representing the three families of amino acid transporters firmly identified (EAAC1, GABA and CAT) are shown in Figure 3.

'Transport-related' proteins

Tate et al. [214] obtained a cDNA clone (NAA, later called NBAA-Tr) which increased the rate of Na^+-independent amino acid transport when expressed in oocytes. It was suggested that this clone was related to System L activity. A clone encoding a protein with an identical amino acid sequence was later isolated by Wells and Hediger [215]. Expression of this clone (D2) in oocytes stimulated the Na^+-independent uptake of both neutral and basic amino acids with a specificity similar to that of System $b^{0,+}$, which is a transporter endogeneously present in oocytes. At the same time, the isolation of another clone (rBAT) from rabbit kidney was reported [216]. This had 79% identity with D2 and also induced System $b^{0,+}$ activity in oocytes. The rBAT protein had similarities to the human surface antigen 4F2. Injection of the cRNA for 4F2 into oocytes stimulated the endogenous

System y^+ activity [217]. The proteins encoded by the clones D2 and rBAT and the 4F2 protein each have only one transmembrane helix and are glycosylated. As all other known mammalian substrate transporters have multiple transmembrane helices, it is unlikely that these proteins are themselves transporters. It has been suggested that they may act as components, e.g. regulatory subunits or activators, of the transport proteins. Antibodies against the NBAA-Tr protein helped to determine its ultrastructural localization in rat kidney and intestine [218,219].

Aminopeptidase N, which has been postulated to interact with System B^0 in bovine brush-border membranes [177], also has a single transmembrane region and is glycosylated. It may be speculated that this enzyme is another in this family of transport-activator proteins. In addition to proteins which appear to stimulate transporters, channel-activator proteins have also been described. The protein IsK acts as an activator of endogenous K^+ and Cl^- channels when expressed in oocytes [220].

Subunit composition of amino acid transport systems

Determination of the amino acid sequences of putative transport proteins from cDNA sequences does not in itself give information about their subunit composition. Radiation inactivation has been used to a limited extent to explore the sizes of transporters *in situ*. Using rat kidney cortex brush-border membrane vesicles, Beliveau et al. [221] observed an apparent molecular mass for the functional Na^+-dependent alanine transporter of 274 kDa. The same value was obtained for leucine transport and presumably relates to the activity of the broad-specificity transporter System B^0. The uptake of lysine via System y^+ was associated with a species of molecular mass 90 kDa, while that of β-alanine was associated with a species of molecular mass 127 kDa. The ecotropic virus receptor (System y^+) has a molecular mass value of 67 kDa while that of the ($Na^+ + Cl^-$)-dependent taurine transporter derived from the cDNA sequence is 74 kDa. The protein responsible for System B^0 activity has not yet been identified, but putative cDNA clones for Na^+-dependent neutral amino acid transport activity (SATT and ASC1) predict proteins with 529 and 532 amino acids respectively. The large discrepancy between the molecular mass of the predicted subunit and the apparent value for the functional transporter for Na^+-dependent neutral amino acid transport suggests that the transporter is composed of multiple subunits or is associated with other proteins. This may not be the case for System y^+, while the β-amino acid transporter may represent an intermediate case. In Ehrlich ascites cells, the apparent molecular size of the protein responsible for Na^+-dependent AIB transport was 347 kDa, again suggesting the involvement of multiple subunits or other proteins [222]. The IMINO transport system of the intestine has been tentatively identified as a species of subunit 95–110 kDa on reducing SDS/polyacrylamide gels [223]. Radiation inactivation studies indicate a functional molecular mass of 382 kDa (see ref. [20] suggesting the possible occurrence of a tetramer in the intact membrane). Similar radiation inactivation studies suggest that the Na^+–glucose symporter has a functional size of 290 kDa and is composed of four subunits [224].

Regulation of tissue mRNA levels for transport proteins

To date, there have been few studies on the regulation of amino acid transport activity at the transcriptional level. It was recently reported [225] that the activity of the X_{AG}^- transport system in the renal epithelial cell line NBL-1 was increased on amino acid deprivation in a cycloheximide-sensitive process. Probes derived from the EAAC1 clone hybridized to a single 3.5 kb transcript in these cells, but the level of the transcript initially decreased rather than increased on induction of transport activity. This may suggest that in this cell line initial induction of glutamate transport is regulated at the post-transcriptional level. It also implies that a search for transport systems induced by amino acid deprivation using subtractive cDNA libraries may not necessarily be fruitful. In contrast, as mentioned previously, mRNA levels for the osmotically regulated *myo*-inositol transporter from MDCK cells [226] are greatly increased on exposure of cells to hyperosmotic medium, suggesting transcriptional regulation of transport in this case.

CONCLUDING REMARKS AND FUTURE PROSPECTS

The study of amino acid transport has been given a new impetus by the recent work on the identification and sequencing of transport proteins outlined above, and the field is now rapidly expanding. A number of interesting findings have emerged. One is the possibility that more than one gene product may correspond to a single kinetically defined transport system and tissue-specific isoforms of transport proteins may also occur. Direct evidence that a cloned cDNA codes for a product that, when expressed heterologously, is really consistent with physiological activity is necessary before assigning a clone to a particular kinetically defined transport activity. Elucidation of the role of identified, and as yet still unidentified, transport-associated proteins in the regulation of amino acid transport is also necessary in order to obtain a complete understanding of how these systems operate at the molecular level.

The availability of molecular probes should now allow the study of the regulation of transport at the transcriptional and translational levels, in a parallel way to published studies on the mammalian glucose transporters. Structural and functional analysis should help us to understand the molecular basis of the broad specificity of these amino acid transport systems. An unexpected aspect of the structural data so far obtained is the apparent close similarity of the proteins catalysing the Na^+-dependent transport of glucose, nucleosides, inositol and neutral amino acids (SAAT1). On the other hand, other putative neutral amino acid transporters (SATT and ASCT1) resemble the glutamate transporters, which are unrelated to the glucose transporter. The relatively small molecular differences which result in these widely divergent substrate specificities will be of great interest.

Finally, the molecular basis for tissue- and development-dependent expression of transporters and their regulation will be of importance in the elucidation of the probable role of amino acid transport systems in many pathophysiological conditions, such as diabetes, neoplasia and amino aciduria. Particular attention will be paid to the molecular events involved in the regulation of the inducible transport System A outlined in this review.

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REFERENCES

1 Christensen, H. N. (1990) Physiol. Rev. **70**, 43–77

2 Kilberg, M. S., Stevens, B. R. and Novak, D. A. (1993) Annu. Rev. Nutr. **13**, 137–165
3 Kilberg, M. S. and Häussinger, D. (eds.) (1992) Mammalian Amino Acid Transport, Plenum Press, New York
4 Oxender, D. L. and Christensen, H. N. (1963) J. Biol. Chem. **238**, 3686–3699
5 Christensen, H. N., Oxender, D. L., Liang, M. and Vatz, K. A. (1965) J. Biol. Chem. **240**, 3609–3616
6 Christensen, H. N. (1985) J. Membr. Biol. **84**, 97–103
7 Christensen, H. N. (1989) Methods Enzymol. **173**, 564–575
8 McGivan, J. D. (1992) in Mammalian Amino Acid Transport (Kilberg, M. S. and Häussinger, D., eds.), pp. 51–64, Plenum Press, New York
9 Heinz, E. (1972) in Metabolic Pathways: Metabolic Transport (Hokin, L. E., ed.), 3rd edn., pp. 455–501, Academic Press, New York
10 Christensen, H. N., Liang, M. and Archer, E. G. (1967) J. Biol. Chem. **242**, 5237–5242
11 Dall'Asta, V., Gazzola, G. C., Franchi-Gazzola, R., Bussolati, O., Longo, N. and Guidotti, G. G. (1983) J. Biol. Chem. **258**, 6371–6379
12 Ferkany, J. and Coyle, J. T. (1986) J. Neurosci. Res. **16**, 491–503
13 Joseph, S. K., Bradford, N. M. and McGivan, J. D. (1978) Biochem. J. **176**, 827–836
14 Kilberg, M. S., Handlogten, M. E. and Christensen, H. N. (1980) J. Biol. Chem. **255**, 4011–4019
15 Hundal, H. S., Rennie, M. J. and Watt, P. W. (1987) J. Physiol. (London) **393**, 283–305
16 Eavenson, E. and Christensen, H. N. (1967) J. Biol. Chem. **242**, 5386–5396
17 Miyamoto, Y., Nakamura, H., Hoshi, T., Ganapathy, V. and Leibach, F. H. (1990) Am. J. Physiol. **259**, G372–G379
18 Stevens, B. R., Kaunitz, J. D. and Wright, E. M. (1984) Annu. Rev. Physiol. **46**, 417–433
19 Maenz, D. D. and Patience, J. F. (1992) J. Biol. Chem. **267**, 22079–22086
20 Stevens, B. R. (1992) in Mammalian Amino Acid Transport (Kilberg, M. S. and Häussinger, D., eds.), pp. 149–163, Plenum Press, New York
21 Lynch, A. M. and McGivan, J. D. (1987) Biochim. Biophys. Acta **899**, 176–184
22 Doyle, F. A. and McGivan, J. D. (1992) Biochim. Biophys. Acta **1104**, 55–62
23 Sepulveda, F. V. and Pearson, J. D. (1982) J. Cell. Physiol. **112**, 182–188
24 Van Winkle, L. J., Christensen, H. N. and Campione, A. L. (1985) J. Biol. Chem. **260**, 12118–12123
25 Shotwell, M. A., Kilberg, M. S. and Oxender, D. L. (1983) Biochim. Biophys. Acta **737**, 267–284
26 Kilberg, M. S., Barber, E. F. and Handlogten, M. E. (1985) Curr. Top. Cell Regul. **25**, 133–163
27 Kilberg, M. S. and Häussinger, D. (1992) in Mammalian Amino Acid Transport (Kilberg, M. S. and Häussinger, D., eds.), pp. 133–148, Plenum Press, New York
28 Eddy, A. A. (1992) in Mammalian Amino Acid Transport (Kilberg, M. S. and Häussinger, D., eds.), pp. 31–48, Plenum Press, New York
29 Eddy, A. A. (1968) Biochem. J. **108**, 195–202
30 Kristensen, L. O. (1980) J. Biol. Chem. **255**, 5236–5243
31 Dall'Asta, V., Bussolati, O., Guidoitti, G. G. and Gazzola, G. C. (1991) J. Biol. Chem. **266**, 1591–1597
32 Geck, P. and Heinz, E. (1976) Biochim. Biophys. Acta **443**, 49–53
33 Edmondson, J. W. and Lumeng, L. (1980) Biochem. Biophys. Res. Commun. **96**, 61–68
34 Moule, S. K. and McGivan, J. D. (1987) Biochem. J. **247**, 233–235
35 Moule, S. K., Bradford, N. M. and McGivan, J. D. (1987) Biochem. J. **241**, 737–743
36 Moule, S. K. and McGivan, J. D. (1990) Eur. J. Biochem. **187**, 677–682
37 Borgese, F., Sardet, C., Cappadoro, M. and Pouyssegur, J. (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 6765–6769
38 Sips, H. J., Groen, H. J. and Tager, J. M. (1980) FEBS Lett. **119**, 271–274
39 McGivan, J. D., Ramsell, J. C. and Lacey, J. H. (1981) Biochim. Biophys. Acta **644**, 295–304
40 Fafournoux, P., Remesy, C. and Demigue, C. (1983) Biochem. J. **210**, 645–652
41 Samson, M., Fehlmann, M., Dolais-Kitabgi, J. and Freychet, P. (1980) Diabetes **29**, 996–1000
42 Barber, E. F., Handlogten, M. E., Vida, T. A. and Kilberg, M. S. (1982) J. Biol. Chem. **257**, 14960–14967
43 Rosenthal, N. R., Jacob, R. and Barrett, E. (1985) Am. J. Physiol. **248**, E581–E587
44 Gumà, A., Castelló, A., Testar, X., Palacín, M. and Zorzano, A. (1992) Biochem. J. **281**, 407–411
45 Fehlmann, M., Le Cam, A., Kitabgi, P., Rey, J. F. and Freychet, P. (1979) J. Biol. Chem. **254**, 401–407
46 Hayes, M. R. and McGivan, J. D. (1982) Biochem. J. **204**, 365–368
47 Kristensen, L. O., Sestoft, L. and Folke, M. (1983) Am. J. Physiol. **244**, G491–G500
48 Pastor-Anglada, M., Remesar, X. and Bourdel, G. (1987) Am. J. Physiol. **252**, E408–E413
49 Felipe, A., Remesar, X. and Pastor-Anglada, M. (1989) Pediatr. Res. **26**, 448–451
50 Bellemann, P. (1981) Biochem. J. **198**, 475–483
51 Leoni, S., Spagnuolo, S., Massimi, M. and Conti Devirgiliis, L. (1990) Membr. Biochem. **9**, 117–128
52 Martínez-Mas, J. V., Casado, J., Felipe, A., Marín, J. J. G. and Pastor-Anglada, M. (1993) Biochem. J. **293**, 819–824
53 Ferrer-Martinez, A., Casado, F. J., Leturque, A., Felipe, A. and Pastor-Anglada, M. (1994) Biochim. Biophys. Acta, in the press
54 Dorio, R. J., Hoek, J. B. and Rubin, E. (1984) J. Biol. Chem. **259**, 11430–11435
55 Fafournoux, P., Rémésy, C. and Demigné, C. (1985) Biochem. J. **231**, 315–320
56 Handlogten, M. E. and Kilberg, M. S. (1982) Biochem. Biophys. Res. Commun. **108**, 1113–1119
57 Dolais-Kitabgi, J., Rey, J. F., Fehlman, M., Kitabgi, P. and Freychet, P. (1981) Endocrinology (Baltimore) **109**, 868–875
58 Reference deleted
59 Fehlmann, M., Le Cam, A. and Freychet, P. (1979) J. Biol. Chem. **254**, 10431–10437
60 Handlogten, M. E. and Kilberg, M. S. (1984) J. Biol. Chem. **259**, 3519–3525
61 Schenerman, M. A. and Kilberg, M. S. (1986) Biochim. Biophys. Acta **856**, 428–436
62 Bracy, D. S., Schenermann, M. A. and Kilberg, M. S. (1987) Biochim. Biophys. Acta **899**, 51–58
63 Cariappa, R. and Kilberg, M. S. (1990) J. Biol. Chem. **265**, 1470–1475
64 Cariappa, R. and Kilberg, M. S. (1992) Am. J. Physiol. **263**, E1021–E1028
65 Canivet, B., Fehlmann, M. and Freychet, P. (1980) Mol. Cell. Endocrinol. **19**, 253–261
66 Kilberg, M. S. (1982) J. Membr. Biol. **69**, 1–12
67 Foster, D. O. and Pardee, A. B. (1969) J. Biol. Chem. **244**, 2675–2681
68 Sander, G. and Pardee, A. B. (1972) J. Cell. Physiol. **80**, 267–272
69 Tramacere, M., Borghetti, A. F. and Guidotti, G. G. (1977) J. Cell. Physiol. **93**, 425–434
70 Oxender, D. L., Lee, M. and Cecchini, G. (1977) J. Biol. Chem. **252**, 2680–2683
71 Borghetti, A. F., Piedimonte, G., Tramacere, M., Severini, A., Ghiringhelli, P. and Guidotti, G. G. (1980) J. Cell. Physiol. **105**, 39–49
72 Amsler, K., Shaffer, C. and Cook, J. S. (1983) J. Cell. Physiol. **114**, 184–190
73 Boerner, P. and Saier, M. H. (1982) J. Cell. Physiol. **113**, 240–246
74 Handlogten, M. E. and Kilberg, M. S. (1988) Am. J. Physiol. **255**, C261–C270
75 Lever, J. E. (1977) J. Biol. Chem. **252**, 1990–1997
76 Leister, K. J., Schenerman, M. A. and Racker, E. (1988) J. Cell. Physiol. **135**, 163–168
77 Walker, R. O. and Whitfield, J. F. (1978) Proc. Natl. Acad. Sci. U.S.A. **75**, 1394–1398
78 Le Cam, A., Rey, J.-F., Fehlmann, M., Kitabgi, P. and Freychet, P. (1979) Am. J. Physiol. **236**, E594–E601
79 Leffert, H. L., Koch, K. S., Lad, P. J., Shapiro, I. P., Skelly, H. and de Hemptinne, B. (1988) in The Liver: Biology and Pathobiology (Arias, I. M., Jakoby, W. B., Popper, H., Schachter, D. and Shafritz, D. A., eds.), 2nd edn., pp. 833–850, Raven Press, New York
80 Fowler, F. C., Banks, R. K. and Mailliard, M. E. (1992) Hepatology **16**, 1187–1194
81 Martinez-Mas, J. V., Ruiz-Montasell, B., Casado, J., Felipe, A. and Pastor-Anglada, M. (1993) FEBS Lett. **329**, 189–193
82 Ruiz-Montasell, B., Casado, F. J., Felipe, A. and Pastor-Anglada, M. (1992) J. Membr. Biol. **128**, 227–233
83 Ruiz-Montasell, B., Martínez-Mas, J. V., Enrich, C., Casado, F. J., Felipe, A. and Pastor-Anglada, M. (1993) FEBS Lett. **316**, 85–88
84 Lu, X. P. and Leffert, H. L. (1991) J. Biol. Chem. **266**, 9276–9284
85 Leoni, S., Spagnuolo, S., Dini, L. and Conti-Devirgilis, L. (1987) J. Cell. Physiol. **130**, 103–110
86 Felipe, A., Remesar, X. and Pastor-Anglada, M. (1993) Metab. Clin. Exp. **42**, 1109–1115
87 Casado, J., Pastor-Anglada, M. and Remesar, X. (1987) Biochem. J. **245**, 297–300
88 Casado, J., Remesar, X. and Pastor-Anglada, M. (1987) Biochem. J. **248**, 117–122
89 Pastor-Anglada, M., Felipe, A., Casado, J. and Remesar, X. (1990) in Endocrine and Biochemical Development of the Fetus and Neonate (Cuezva, J. M., Pascual-Leone, A. M. and Patel, M. S., eds.), pp. 287–290, Plenum Press, New York
90 Chiles, T. C. and Kilberg, M. S. (1986) J. Cell. Physiol. **129**, 321–328
91 Dudeck, K. L., Dudenhausen, E. E., Chiles, T. C., Fafournoux, P. and Kilberg, M. S. (1987) J. Biol. Chem. **262**, 12565–12569
92 Pegg, A. E. (1988) Cancer Res. **48**, 759–774
93 Rinehart, C. A. and Canellakis, E. S. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 4365–4368
94 Rinehart, C. A., Viceps-Madore, D., Fong, W.-F., Ortiz, J. G. and Canellakis, E. S. (1985) J. Cell. Physiol. **123**, 435–441
95 Kanamoto, R., Boyle, S. M., Oka, T. and Hayashi, S. (1987) J. Biol. Chem. **262**, 14801–14805
96 Kristensen, L. O. (1986) Am. J. Physiol. **251**, G575–G584

97 Baquet, A., Hue, L., Meijer, A., van Woerkom, G. M. and Plomp, P. J. A. M. (1990) J. Biol. Chem. **265**, 955–959
98 Häussinger, D., Lang, F., Bauers, K. and Gerok, W. (1990) Eur. J. Biochem. **188**, 689–695
99 Gazzola, G. C., Dall'Asta, V., Nucci, F. A., Rossi, P. A., Bussolati, O., Hoffmann, E. K. and Guidotti, G. G. (1991) Cell. Physiol. Biochem. **1**, 131–142
100 Poulin, R. and Pegg, A. E. (1990) J. Biol. Chem. **265**, 4025–4032
101 Auberger, P., Samon, M., LeCam, G. and LeCam, A. (1983) J. Cell. Physiol. **117**, 204–210
102 Russell, D. and Snyder, S. H. (1968) Proc. Natl. Acad. Sci. U.S.A. **60**, 1420–1427
103 Hitier, Y., Champigny, O. and Bourdel, G. (1983) Am. J. Physiol. **244**, E548–E554
104 Piacentini, M., Sartori, C., Beninati, S., Bargagli, A. M. and Ceru-Argento, M. P. (1986) Biochem. J. **234**, 435–440
105 Tramacere, M., Petronini, P. G., Severini, A. and Borghetti, A. F. (1984) Exp. Cell. Res. **151**, 70–79
106 Klip, A., Mack, E., Cragoe, E. J., Jr. and Grinstein, S. (1986) J. Cell. Physiol. **127**, 244–252
107 Nucci, F. A., Rossi, P. A., Hofmann, E. K., Dall'Asta, V., Bussolati, O., Guidotti, G. G. and Gazzola, G. C. (1990) Renal Physiol. Biochem. **13**, 172–173
108 Soler, C., Felipe, A., Casado, F. J., McGivan, J. D. and Pastor-Anglada, M. (1993) Biochem. J. **289**, 653–658
109 Reference deleted
110 Petronini, P. G., Tramacere, M., Kay, J. E. and Borghetti, A. F. (1986) Exp. Cell. Res. **165**, 180–190
111 Petronini, P. G., Tramacere, M., Mazzini, A., Kay, J. E. and Borghetti, A. F. (1989) J. Cell. Physiol. **140**, 202–211
112 Petronini, P. G., Tramacere, M., Wheeler, K. P. and Borghetti, A. F. (1990) Biochim. Biophys. Acta **1053**, 144–150
113 Petronini, P. G., Tramacere, M., Mazzinin, A., Piedemonte, G., Silvotti, L. and Borghetti, A. F. (1987) Exp. Cell. Res. **172**, 450–462
114 Nelson, W. J. and Hammerton, R. W. (1989) J. Cell Biol. **108**, 893–902
115 Molitoris, B. A. and Nelson, W. J. (1990) J. Clin. Invest. **85**, 3–9
116 Davis, J. Q. and Bennett, V. (1990) J. Biol. Chem. **265**, 17252–17256
117 Cantiello, H. F., Stow, J. L., Prat, A. G. and Ausiello, D. A. (1991) Am. J. Physiol. **261**, C882–C888
118 Shapiro, M., Matthews, J. B., Hehct, G., Delp, C. and Madara, J. L. (1991) J. Clin. Invest. **87**, 1903–1909
119 Matthews, J. B., Awtrey, C. S. and Madara, J. L. (1992) J. Clin. Invest. **90**, 1608–1613
120 Gründer, S., Thiemann, A., Pusch, M. and Jentsch, T. J. (1992) Nature (London) **360**, 759–762
121 Cornet, M., Ubi, J. and Kolb, H.-A. (1993) J. Membr. Biol. **133**, 161–170
122 Roy, G. and Sauvé, R. (1987) J. Membr. Biol. **100**, 83–96
123 Roy, G. and Malo, C. (1992) J. Membr. Biol. **130**, 83–90
124 Sánchez Olea, R., Pasantes-Morales, H., Lázaro, A. and Cereijido, M. (1991) J. Membr. Biol. **121**, 1–9
125 Banderali, U. and Roy, G. (1992) Am. J. Physiol. **263**, C1200–C1207
126 Bode, B. P. and Kilberg, M. S. (1991) J. Biol. Chem. **266**, 7376–7381
127 Robey, R. B., Kwon, H. M., Handler, J. S., Garcia-Perez, A. and Burg, M. B. (1991) J. Biol. Chem. **266**, 10400–10405
128 Yamauchi, A., Uchida, S., Kwon, H. M., Preston, A. S., Robey, R. B., Garcia-Perez, A., Burg, M. B. and Handler, J. S. (1992) J. Biol. Chem. **267**, 649–652
129 Kwon, H. M., Yamauchi, A., Uchida, S., Robey, R. B., Garcia-Perez, A., Burg, M. B. and Handler, J. S. (1991) Am. J. Physiol. **260**, F258–F263
130 Kwon, H. M., Yamauchi, A., Uchida, S., Preston, A. S., García-Pérez, A., Burg, M. and Handler, J. S. (1992) J. Biol. Chem. **267**, 6297–6301
131 Uchida, S., Yamauchi, A., Preston, A. S., Kwon, H. M. and Handler, J. S. (1993) J. Clin. Invest. **91**, 1604–1607
132 Schulz, W. A., Eickelmann, P., Hallbrucker, C., Sies, H. and Häussinger, D. (1991) FEBS Lett. **292**, 264–266
133 Theodoropoulos, P. A., Stournaras, C., Stoll, B., Markogiannakis, E., Lang, F., Gravanis, A. and Häussinger, D. (1992) FEBS Lett. **311**, 241–245
134 Moffett, J. and Englesberg, E. (1984) Mol. Cell. Biol. **4**, 799–808
135 Englesberg, E. and Moffett, J. (1986) J. Membr. Biol. **91**, 199–212
136 Kilberg, M. S. (1986) Trends Biochem. Sci. **11**, 183–186
137 Bracy, D. S., Handlogten, M. E., Barber, E. F., Han, H. P. and Kilberg, M. S. (1986) J. Biol. Chem. **261**, 1514–1520
138 Englesberg, E., Moffett, J. and Périer, F. (1986) Fed. Proc. Fed. Am. Soc. Exp. Biol. **45**, 2441–2443
139 Bass, R., Hedegaard, H. B., Dillehay, L., Moffett, J. and Englesberg, E. (1981) J. Biol. Chem. **256**, 10259–10266
140 Shotwell, M. A., Jayme, D. W., Kilberg, M. S. and Oxender, D. L. (1981) J. Biol. Chem. **256**, 5422–5427
141 Moffett, J. and Englesberg, E. (1986) J. Cell. Physiol. **126**, 421–429
142 Fafournoux, P., Rémésy, C. and Demigné, C. (1990) Am. J. Physiol. **259**, E614–E625
143 Ruiz, B., Felipe, A., Casado, J. and Pastor-Anglada, M. (1991) Biochem. J. **280**, 367–372
144 Van Dyke, R. W. and Scharschmidt, B. F. (1983) J. Biol. Chem. **258**, 12912–12919
145 Johnson, E. and Eddy, A. A. (1985) Biochem. J. **226**, 773–779
146 Zibirre, R., Poronnik, P. and Koch, G. (1986) J. Cell. Physiol. **129**, 85–93
147 Renner, E. L., Lake, J. R., Cragoe, E. J., Jr. and Scharschmidt, B. F. (1988) Biochim. Biophys. Acta **938**, 386–394
148 Cohen, B. J. and Lechene, C. (1990) Am. J. Physiol. **258**, C24–C29
149 Bourdel, G., Forestier, M. and Gouhot, B. (1990) Biochim. Biophys. Acta **1026**, 1–12
150 Le Cam, A., Rey, J.-F., Fehlmann, M., Kitabgi, P. and Freychet, P. (1979) Am. J. Physiol. **236**, E594–E602
151 Reference deleted
152 Blostein, R. and Grafova, E. (1987) Biochem. Cell. Biol. **65**, 869–875
153 Mills, B. and Tupper, J. T. (1976) J. Cell. Physiol. **89**, 123–132
154 Mager, D. and Bernstein, A. (1978) J. Cell. Physiol. **94**, 275–286
155 Graves, J. S. and Wheeler, D. D. (1982) Am. J. Physiol. **243**, C124–C132
156 Johnson, M. D., Ash, J. F. and Pauw, P. G. (1986) J. Biol. Chem. **261**, 40–43
157 Schenerman, M. A., Leister, K. J., Trachtenberg, D. K. and Racker, E. (1988) J. Cell. Physiol. **135**, 157–162
158 Leister, K. J., Schenerman, M. A. and Racker, E. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 783–786
159 Qian, N. X. (1991) Ph.D. Thesis, University of California, Santa Barbara, CA
160 Qian, N. X., Jones, M., McDonough, A. and Englesberg, E. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 7984–7988
161 Qian, N. X., Pastor-Anglada, M. and Englesberg, E. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 3416–3420
162 Koepsell, H., Korn, K., Ferguson, D., Menhur, H., Ollig, D. and Haase, W. (1984) J. Biol. Chem. **259**, 6548–6558
163 Fafournoux, P., Dudenhausen, E. E. and Kilberg, M. S. (1989) J. Biol. Chem. **264**, 4805–4811
164 Quesada, A. and McGivan, J. D. (1988) Biochem. J. **255**, 963–969
165 Tamarapoo, B. K. and Kilberg, M. S. (1991) Biochem. J. **274**, 97–101
166 Doyle, F. A. and McGivan, J. D. (1992) Biochem. J. **281**, 95–102
167 Radian, R., Bendahan, A. and Kanner, B. I. (1986) J. Biol. Chem. **261**, 15437–15441
168 Guastella, J., Nelson, N., Nelson, H., Czyzyk, L., Keynan, S., Miedel, M. C., Davidson, N., Lester, H. A. and Kanner, B. I. (1990) Science **249** 1303–1306
169 Danbolt, N. C., Pines, G. and Kanner, B. I. (1990) Biochemistry **29**, 6734–6740
170 Pines, G., Danbolt, N. C., Bjoras, M., Zhang, Y., Bendahan, A., Eide, L., Koepsell, H., Storm-Mathison, J., Seeberg, E. and Kanner, B. (1992) Nature (London) **360**, 464–467
171 Storck, T., Schulte, S., Hofman, K. and Stoffel, W. (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 10955–10959
172 Klockner, U., Storck, T., Conradt, M. and Stoffel, W. (1993) J. Biol. Chem. **268**, 14594–14596
173 Lopez-Corcuera, B., Vazquez, J. and Aragon, C. (1991) J. Biol. Chem. **266**, 24809–24814
174 Lopez-Corcuera, B., Alcantara, R., Vazquez, J. and Aragon, C. (1993) J. Biol. Chem. **268**, 2239–2243
175 McCormick, J. I. and Johnstone, R. M. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 7877–7881
176 Tamarapoo, B. K., Handlogten, M. E., , Laine, R. O., Serrano, M. A., Dugan, J. and Kilberg, M. S. (1991) J. Biol. Chem. **267**, 2370–2374
177 Plakidou-Dymock, S., Tanner, M. J. A. and McGivan, J. D. (1993) Biochem. J. **290**, 59–65
178 Plakidou-Dymock, S. and McGivan, J. D. (1993) Biochim. Biophys. Acta **1145**, 105–112
179 Hediger, M. A., Coady, M. J., Ikada, T. S. and Wright, E. M. (1987) Nature (London) **330**, 379–381
180 Coady, M. J., Pajor, A. M., Toloza, E. M. and Wright, E. M. (1990) Arch. Biochem. Biophys. **283**, 130–134
181 Aoshima, H., Tomita, K. and Sugio, S. (1988) Arch. Biochem. Biophys. **265**, 73–81
182 Tate, S. S., Urade, R., Getchell, T. V. and Udenfriend, S. (1989) Arch. Biochem. Biophys. **275**, 591–596
183 McNamara, P. D., Rea, C. T. and Segal, S. (1991) J. Biol. Chem. **266**, 986–989
184 Su, T.-Z., Logsdon, C. G. and Oxender, D. L. (1992) Mol. Cell. Biol. **12**, 5281–5287
185 Palacin, M., Werner, A., Dittmer, J., Murer, H. and Biber, J. (1990) Biochem. J. **270**, 189–195
186 Tarnuzzer, R. W., Campa, M. J., Qian, N. X., Englesberg, E. and Kilberg, M. S. (1990) J. Biol. Chem. **265**, 13914–13917
187 Taylor, P. M., Mackenzie, B., Low, S. Y. and Rennie, M. J. (1992) J. Biol. Chem. **267**, 3873–3877

188 Kim, J. W., Closs, E. I., Albritton, L. M. and Cunningham, J. M. (1991) Nature (London) **352**, 725–728
189 Wang, H., Kavanaugh, M. P., North, R. A. and Kabat, D. (1991) Nature (London) **352**, 729–731
190 Albritton, L. M., Tseng, L., Scadden, D. and Cunningham, J. M. (1989) Cell **57**, 659–666
191 Wang, H., Dechant, E., Kavanaugh, M., North, R. A. and Kabat, D. (1992) J. Biol. Chem. **267**, 23617–23624
192 Albritton, L. M., Kim, J. W., Tseng, L. and Cunningham, J. M. (1993) J. Virol. **67**, 2091–2096
193 Closs, E. I., Albritton, L. M., Kim, J. W. and Cunningham, J. M. (1993) J. Biol. Chem. **268**, 7538–7544
194 Closs, E. I., Rinkes, I. H. M. B., Bader, A., Yarmush, M. L. and Cunningham, J. M. (1993) J. Virol. **67**, 2097–2102
195 Kanai, Y. and Hediger, M. A. (1992) Nature (London) **360**, 467–471
196 Kanner, B. I. (1993) FEBS Lett. **325**, 95–99
197 Kanai, Y., Smith, C. P. and Hediger, M. A. (1993) Trends Neurosci. **16**, 365–370
198 Pacholczyk, T., Blakely, R. D. and Amara, S. G. (1991) Nature (London) **350**, 350–354
199 Giros, B., Mestikowsky, S. E., Bertrand, L. and Caron, M. G. (1991) FEBS Lett. **295**, 149–154
200 Shimada, S., Kitayama, S., Lin, C.-L., Patel, A., Nanthakumar, E., Gregor, P., Kuhar, M. and Uhl, G. (1991) Science **254**, 576–578
201 Kilty, J. E., Lorang, D. and Amara, S. G. (1991) Science **254**, 578–579
202 Hoffman, B. J., Mezey, E. and Brownstein, M. J. (1991) Science **254**, 579–580
203 Fremeau, R. T., Caron, M. G. and Blakely, R. D. (1992) Neuron **8**, 915–926
204 Guastella, J., Brecha, N., Weigmann, C., Leaster, H. A. and Davidson, N. (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 7189–7193
205 Smith, K. E., Borden, L. A., Hartig, P. R., Branchek, T. and Weinshank, R. C. (1992) Neuron **8**, 927–935
206 Liu, Q.-R., Nelson, H., Mandiyan, S., Lopez-Corcuera, B. and Nelson, N. (1992) FEBS Lett. **305**, 110–114
207 Uchida, S., Kwon, H. M., Yamauchi, A., Preston, A. S., Marumo, F. and Handler, J. S. (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 8230–8234
208 Liu, Q.-R., Lopez-Corcuera, B., Nelson, H., Mandiyan, S. and Nelson, N. (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 12145–12149
209 Amara, S. G. and Pacholczyk, T. (1991) Curr. Opin. Neurobiol. **1**, 84–90
210 Schloss, P., Mayser, W. and Betz, H. (1992) FEBS Lett. **307**, 76–80
211 Kong, C.-T., Yet, S.-F. and Lever, J. E. (1993) J. Biol. Chem. **268**, 1509–1512
212 Shafqat, S., Tamarapoo, B. K., Kilberg, M. S., Puranam, R. S., McNamara, J. O., Guadano-Ferraz, A. and Fremeau, M. T. (1993) J. Biol. Chem. **268**, 15351–15355
213 Arriza, J. L., Kavanaugh, M. P., Fairman, W. A., Wu, Y.-N., Murdoch, G. H., North, R. A. and Amara, S. G. (1993) J. Biol. Chem. **268**, 15329–15332
214 Tate, S. S., Yan, N. and Udenfriend, S. (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 1–5
215 Wells, R. G. and Hediger, M. A. (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 5596–5600
216 Bertran, J., Werner, A., Moore, M. L., Strange, G., Markovitch, D., Biber, J., Testar, X., Zorzano, A., Palacin, M. and Murer, H. (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 5601–5605
217 Bertran, J., Magagnin, S., Werner, A., Markovitch, D., Biber, J., Testar, X., Zorzano, A., Kuhn, L. C., Palacin, M. and Murer, H. (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 5606–5610
218 Mosckovitz, R., Yan, N., Heimer, E., Felix, A., Tate, S. S. and Udenfriend, S. (1993) Proc. Natl. Acad. Sci. U.S.A. **90**, 4022–4026
219 Pickel, V. M., Nirenberg, M. J., Chan, J., Mosckovitz, R., Udenfriend, S. and Tate, S. S. (1993) Proc. Natl. Acad. Sci. U.S.A. **90**, 7779–7783
220 Attall, B., Guillemare, E., Lasage, F., Honore, E., Romey, G., Lazdunski, M. and Barhanin, J. (1993) Nature (London) **365**, 850–855
221 Beliveau, R., Demeule, M., Jette, M. and Potier, M. (1990) Biochem. J. **268**, 195–200
222 McCormick, J. I., Jette, M., Potier, M., Beliveau, R. and Johnstone, R. M. (1991) Biochemistry **30**, 3704–3709
223 Wright, E. M. and Peerce, B. E. (1984) J. Biol. Chem. **259**, 14993–14998
224 Stevens, B. R., Fernandez, A., Hirayama, B., Wright, E. M. and Kempner, E. S. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 1456–1462
225 Plakidou-Dymock, S. and McGivan, J. D. (1993) Biochem. J. **295**, 749–755
226 Yamauchi, A., Uchida, S., Preston, A. S., Kwon, H. A. and Handler, J. S. (1993) Am. J. Physiol. **264**, F20–F23