Tertiary active transport of amino acids reconstituted by coexpression of System A and L transporters in *Xenopus* oocytes

Fiona E. Baird,1 Kevin J. Bett,1 Catherine MacLean,1 Andrew R. Tee,2 Harinder S. Hundal,1 and Peter M. Taylor1

1Division of Molecular Physiology, College of Life Sciences, University of Dundee, Dundee; and 2Institute of Medical Genetics, Wales College of Medicine, Cardiff University Heath Park, Cardiff, United Kingdom

Submitted 20 May 2009; accepted in final form 17 July 2009

Baird FE, Bett KJ, MacLean C, Tee AR, Hundal HS, Taylor PM. Tertiary active transport of amino acids reconstituted by coexpression of System A and L transporters in *Xenopus* oocytes. *Am J Physiol Endocrinol Metab* 297: E822–E829, 2009.—The System L transporter facilitates cellular import of large neutral amino acids (AAs) such as Leu, a potent activator of the intracellular target of rapamycin (TOR) pathway, which signals for cell growth. System L is an AA exchanger, proposed to accumulate certain AAs by coupling to dissipation of concentration gradient(s) of exchange substrates generated by secondary active AA transporters such as System A (SNAT2). We addressed the hypothesis that this type of coupling (termed tertiary active transport) acts as an indirect mechanism to extend the range of AA stimulating TOR to those transported by both Systems A and L (e.g., Gln) through downstream enhancement of Leu accumulation. System A overexpression enabled *Xenopus* oocytes to accumulate substrate AAs (notably Ser, Gln, Ala, Pro, Met; totaling 2.6 nmol/oocyte) from medium containing a physiological AA mixture at plasma concentrations. Net accumulation of System L (4F2hc-xLAT1) substrates from this medium by System L-overexpressing oocytes was increased by 90% (from 0.7 to 1.35 nmol/oocyte; mainly Leu, Ile) when Systems A and L were coexpressed, coincident with a decline in accumulation of specific System A substrates (Gln, Ser, Met), as expected if the latter were also System L substrates and functional coupling of the transport Systems occurred. AA flux coupling was confirmed as *trans*-stimulation of Leu influx in System L-expressing oocytes by Gln injection (0.5 nmol/oocyte). The observed changes in Leu accumulation are sufficient to activate the TOR pathway in oocytes, although intracellular AA metabolism limits the potential for AA accumulation by tertiary active transport in this system.

nutrient transport; target of rapamycin pathway; glutamine; leucine; amino acid exchanger

AMINO ACIDS (AAs) act through several nutrient signaling cascades to modulate processes, including global protein synthesis, proteolysis, and mRNA turnover, as well as the transport and metabolism of AAs themselves (e.g., see Refs. 1 and 18 for review). These signaling cascades, which include the extensively studied target of rapamycin (TOR) and (GCN) pathways, are regulated by mechanisms that generally involve upstream sensing of intracellular AA concentrations (see Refs. 13 and 14 for review). “Steady-state” intracellular AA concentration are established by the balance of processes of protein and free-AA turnover and the selective permeability properties of the cell surface (including transport and diffusion pathways). Inhibitor and gene-silencing studies have demonstrated a key role for particular AA transporters in the maintenance of free AA concentrations in animal cells (11, 16, 23), implying that signaling pathways regulated by intracellular AA concentration may be intrinsically linked to AA transporter activity.

Indispensable AA (notably leucine) added to the extracellular medium are potent activators of the nutrient-sensitive TOR pathway signaling for growth of animal cells (see Refs. 1 and 10 for review). The plasma membrane transport system L is the only efficient pathway for import of large neutral AAs such as leucine and phenylalanine in many cell types, making it a potentially important step for regulation of TOR pathway activity. System L transporters are composed of heterodimers of an AA permease (SLC7A5 or SLC7A8; LAT1 and -2, respectively) and the 4F2hc (SLC3A2) glycoprotein (31). The LAT1-type transporter is predominantly expressed in tissues with high rates of growth and protein turnover (including many proliferating tumors) (10, 24, 28) and is therefore of particular interest in the context of TOR signaling. It is an obligatory AA exchanger with 1:1 stoichiometry, proposed to accumulate AAs intracellularly by coupling to dissipation of the concentration gradient of exchange substrates (21, 23, 31). Such gradients are generally assumed to be generated by activity of secondary active AA transporters with overlapping AA selectivity, such as System A (5, 14, 21). System A transporters mediate the concentrative uptake of small neutral amino acids, coupled to the uptake of Na+ with a stoichiometry of 1:1 and have a ubiquitous tissue distribution. SNAT2 (SLC38A2) is the predominant System A isoform in most extraneural tissues (20) and appears to be the most widespread major regulated isoform in response to endocrine and nutrient stimuli (15, 16, 18, 35).

System A- and System L-type transporters are coexpressed in the majority of mammalian cell types, and their functional cooperation in terms of propagation of solute gradients via antiport is generally assumed to occur (5, 14, 31, 32; see also Fig. 1), although supporting experimental evidence stems largely from classical studies in which changes of intracellular AA were inferred from tracer equilibration studies (ignoring possible effects of metabolism) or induced by AA preloading and used cultured cell lines now known to express a broad variety of AA transport systems (see, e.g., Refs. 5, 25, and 31). In the present study, we examine this type of coupling, termed tertiary active transport, in more detail using physiologically relevant extracellular AA concentrations and an experimental system (the *Xenopus* oocyte) in which both secondary and (putative) tertiary active AA transporters were overexpressed at the cell surface (Fig. 1). We have shown previously (6) that the *Xenopus* oocyte has a sensing mechanism for intracellular AAs upstream of TOR, which appears to be directly responsive to leucine and several other large neutral AAs (e.g., phenylal-
Fig. 1. Integration of primary (I), secondary (II), and tertiary (III) active transport mechanisms helps determine the trans-membrane distribution of neutral amino acids (AAs) in animal cells. Using the Na⁺-electrochemical gradient established by primary active transport (Na⁺/K⁺ pump), secondary active transporters (e.g., System A) generate net movement of neutral AA substrates from extracellular to intracellular pool, whereas downstream coupling of AA flows by antiport through exchangers such as System L generates tertiary active transport and allows for redistribution of individual AAs without affecting total pool sizes (32). In the present study, we overexpress System A and/or System L in Xenopus oocytes, which express endogenous Na⁺/K⁺ pump activity (27).

anine) but not to glutamine, alanine, or glutamate. Nevertheless, there are reports that dispensable AA such as glutamine exert similar (34) [or sometimes opposing (9, 22)] effects to leucine on TOR pathway activity or are at least required for the leucine-induced effect (12, 23). Nicklin et al. (23) have recently shown that, in amino acid-depleted HeLa cells, glutamine uptake by the high-affinity SLC1A5 (ASCT2; System ASC) transporter primes leucine uptake through System L (SLC7A5/SLC3A2) and subsequent TOR pathway activation. We therefore focused particularly on the ability of glutamine (a substrate for both Systems A and L) to generate tertiary active AA transport and cellular accumulation of TOR pathway activators such as leucine.

EXPERIMENTAL PROCEDURES

Materials. Female toads (Xenopus laevis) were obtained from the South African Xenopus facility (Noordhoek, South Africa). Collagenase A was purchased from Roche Diagnostics (Mannheim, Germany). Radiotracer solutions were obtained from NEN (PerkinElmer Life Sciences, Cambridge, UK). All other chemicals were obtained from Sigma (Poole, UK). Plasmid DNA containing rat SNAT2 cDNA was linearised with HincII (pTTLN2- rat SNAT2) (35), and cRNA was synthesized in vitro using the SP6 mMessageMachine kit (Ambion, Austin, TX) and then purified by phenol-chloroform-isoamyl alcohol extraction. cDNAs encoding IU12 and human 4F2hc were subcloned into the multiple-cloning region of pSG5 (an SV-40 driven expression plasmid) as described previously (26). The Xenopus System L transporter IU12 resembles mammalian LAT1 in preferring large neutral AAs, which is not recognized as a substrate and having relatively high affinities for substrates (26). In this paper, we refer to IU12 as xLAT1. Oocytes were injected with cRNA, cDNA, or AA solution using glass micropipettes connected to a positive displacement microinjector (World Precision Instruments, Sarasota, FL).

Isolation of oocytes and cRNA/cDNA expression. Stage V-VI (prophase-arrested) oocytes from Xenopus laevis were isolated and maintained at 18°C in modified Barth’s medium (MBM) and injected with 50 ng of SNAT2 cRNA (at 1 ng/ml Ultraspec water) into the cytoplasm and/or 2 ng each of 4F2hc/xLAT1 DNA in 15 nl of Ultraspec water into the nucleus, as described previously (3, 26); control oocytes were injected with Ultraspec water only. When studies were undertaken of coexpressed Systems A and L, SNAT2 cRNA was injected 18 h prior to 4F2hc/xLAT1 cDNAs to improve oocyte survival and functional expression of both transporters. Oocytes were incubated for 2–3 days to allow full expression of the transport activity before experiments were performed.

AA transport assays. Influx of radiolabeled AA tracer in Xenopus oocytes was measured as follows. System A transport activity was measured using 0.05 mM [14C]methylaminoisobutyric acid (MeAIB) in 100 mM NaCl transport buffer, pH 8.0 (3), and System L transport activity using 0.01 mM l-[3H]phenylalanine in Na⁺-free (100 mM tetramethylammonium chloride) transport buffer, pH 7.5 (26). Individual measurements on 8–11 oocytes were made for each uptake experiment. Efflux of radiolabeled AA tracer injected into oocytes (50 nl l-[3H]glutamine) was measured as described previously (2).

Amino acid tracer metabolism. l-[3H]glutamine or l-[3H]leucine tracer solution (50 nl) was injected into individual oocytes, which were allowed 10 min for recovery and then transferred in groups of five to 1 ml of fresh MBM and either processed immediately (0 time point) or after 2- or 4-h incubation periods. Oocytes were processed as follows. They were rinsed, homogenized in 20 μl of ice-cold water, and deproteinized by addition of 25 μl 20% PCA, followed by centrifugation for 30 min (4°C at 10,000 rpm). The supernatant was neutralized with 20 μl of 2M potassium carbonate and centrifuged; a 20-μl aliquot of the resulting supernatant was taken directly for scintillation counting, and a second aliquot was dried overnight (Speedvac vacuum dryer) and redisolved in 20 μl of water prior to scintillation counting. Aliquots of the MBM incubation medium were treated similarly. The oocyte protein pellet was washed in 50 μl of 20% PCA, repelleted by centrifugation, and dissolved in 20 μl of 0.2 M NaOH/0.1% SDS prior to scintillation counting. The extent of tracer oxidation was estimated from the amount of volatile radioactive tracer (indicating catabolism to 3H2O) evaporated off during the drying process, expressed as a proportion of the total radioactivity recovered from oocytes (including that in protein) and medium.

HPLC analysis of sample AA concentrations. Free AA concentrations were determined by reverse-phase HPLC with post-column UV (254 nm) detection, following pre-column sample derivatization with phenylisothiocyanate (PITC) as described previously (2).

Presentation of results. Results are expressed as mean values ± SE for n measurements or experiments. To test for statistical significance, the difference between mean values was assessed using Student’s unpaired t-test, with significance assigned at P < 0.05. Where multiple comparisons were required, analysis of variance was performed, and differences were determined using least significant difference.

RESULTS

The absolute levels of System A (SNAT2) and System L (4F2hc-xLAT1) transport activity achieved by overexpression in Xenopus oocytes were the same for single- and dual-transporter-expressing oocytes from an individual batch (Fig. 2A), although the overall expression levels did differ slightly between batches. Glutamine is a substrate for both System L (Fig. 2B) and System A (2) in this experimental system, whereas leucine is a substrate for System L only (21, 31). We confirmed direct coupling of glutamine and leucine fluxes in System L-expressing oocytes by showing 1) that glutamine efflux is trans-stimulated by external leucine (Fig. 3A) and 2) that leucine influx is trans-stimulated by prior injection of glutamine (0.5 nmol/oocyte) but not by the System A-selective MeAIB, which is not a substrate for System L (Fig. 3B). AA efflux from oocytes is unaffected by expression of SNAT2 or 4F2hc-xLAT1 in the absence of extracellular AA (Fig. 3A; Ref. 2).
Intra-oocyte AA concentrations were largely unaffected by overexpression of AA transporters in standard MBM culture. System A-overexpressing oocytes showed substantial time-dependent increases in intracellular concentrations of Gln (Fig. 4), Ala, Met, Asn, Ser, Gly, His, Thr, and Pro (totaling 2.6 nmol/oocyte at steady state; Fig. 5, A and B) when incubated in MBM containing an AA mixture at physiological plasma concentrations, whereas System L overexpression led to smaller increases of Leu (Fig. 4), Ile, Val, Tyr, Trp, and Phe (totaling 0.7 nmol/oocyte; Fig. 5, A and B). These changes in steady-state AA distribution were complete within 5 h of transfer to MBM containing AA mixture (Fig. 4) and are broadly consistent with preferred substrate specificities of the different transporters. AAs not recognized as substrates for either System A or L (notably Glu, Asp, Arg, Lys) showed no significant changes in concentration under any circumstance (data not shown). In contrast, control oocytes showed negligible increases in AA concentrations when supplemented with physiological levels of [AA] externally and, for several AAs (including Leu and Gln), maintained substantially lower intracellular concentrations than in the surrounding medium (Fig. 5A), suggestive of significant rates of intracellular catabolism and/or sequestration into protein. We were indeed able to measure metabolism of both leucine and glutamine over experimental

![Fig. 2. A: Influx of methylaminoisobutyric acid (MeAIB) and phenylalanine in oocytes overexpressing System A and/or System L transporters. Influx of 0.05 mM [14C]MeAIB (System A substrate) or 0.01 mM L-[3H]phenylalanine (System L substrate) was measured over 30 min. Representative results are presented as means ± SE for 11–14 oocytes from one batch. B: glutamine influx into System L-overexpressing oocytes as a function of external AA concentration. Oocytes expressing System L (4F2hc-xLAT1) or controls were incubated in transport buffer containing 0.01–5 mM L-[3H]glutamine. Each point represents mean influx ± SE for 9–11 oocytes from one batch. To estimate $K_m$ and $V_{max}$ for System L, influx values for control oocytes were subtracted from corresponding values for System L-expressing oocytes, and data were analyzed by fitting of a hyperbola using nonlinear regression procedures (GraphPad Prism 4). Calculated values for glutamine influx, $K_m = 0.8 \pm 0.15$ mM; $V_{max} = 720 \pm 45$ pmol·oocyte$^{-1}$·h$^{-1}$.

![Fig. 3. Trans-stimulation of Leu-Gln heteroexchanges through System L (4F2hc-xLAT1). A: representative result showing efflux of injected L-[3H]glutamine tracer from oocytes (System L expressing and controls) into transport buffer in absence (basal) or presence of 2 mM leucine. Results are presented as mean efflux rate constant ± SE for 7–8 oocytes. *P < 0.05 from respective control value. Similar results were obtained in a separate experiment using a different oocyte batch. B: influx of 0.01 mM L-[3H]leucine in System L-expressing oocytes preinjected with 50 nl of water (basal) or solution containing 500 pmol glutamine or MeAIB. Values shown are means ± SE for 3 batches of oocytes. *P < 0.05 from water value.

![Fig. 4. Time course of net accumulation of glutamine (circles) and leucine (triangles) by oocytes from modified Barth's medium (MBM) containing an AA mixture at nominal physiological plasma concentrations (Ref. 29; including 0.67 mM Gln and 0.16 mM Leu) over the indicated incubation periods. Glutamine values are from oocytes overexpressing System A (●) or controls (○), whereas leucine values are from oocytes overexpressing System L (▲) or controls (△). Each point represents the mean ± SE value for triplicate measurements in a representative batch of oocytes.
periods (Fig. 6), indicating that oocytes catabolize AA in significant amounts.

Net accumulation of System L substrates (mainly Leu and Ile) was enhanced by 90% overall (to 1.35 nmol/oocyte) when Systems A and L were coexpressed (Fig. 5, A and B), coincident with a decline in accumulation of certain System A substrates (principally Gln, Met, Ser), as might be predicted if these latter AAs were also System L substrates and functional coupling of the transporters had dissipated their concentration gradients. Consistent with this concept, a similar pattern of changes in oocyte AA concentrations emerged when they were preloaded with glutamine (5 mM overnight) and then exposed to 5 mM leucine for 4 h (Fig. 7). The substantial accumulation of alanine and proline by System A-expressing oocytes from MBM containing the AA mix was unaffected by coexpression of System L, consistent with a known lack of interaction with LAT1-type System L activity (21, 31).

Neither the reciprocal changes in Gln:Leu concentration shown in Fig. 7 (230:150 pmol/oocyte) nor the equivalent totaled changes in System A and System L substrate concentrations shown in Fig. 5B match exactly (as might be expected if driven solely by a 1:1 heteroexchange mechanism), with the decline in System A substrates being in slight excess. This might be in part explained by a significant contribution of AA metabolism to the steady-state AA distributions; therefore, we investigated the possibility that changes in intracellular leucine concentration were influenced by altered rates of leucine catabolism resulting from intracellular accumulation of other AAs. Glutamine injection into oocytes (0.5 nmol/oocyte) produced a marked stimulation of intracellular leucine catabolism (from 2.7 to 10.3%/h; Fig. 6), from which we infer that we may be underestimating the extent to which tertiary active transport might accumulate leucine in our experimental system. Metabolism of glutamine itself did not appear to be significantly stimulated by increased intracellular AA concentrations.

The observation that System A-expressing oocytes incubated in 5 mM glutamine show no greater accumulation of this AA than do those in a physiological mixture containing 0.67 mM glutamine (compare Figs. 5A and 7A) was unexpected, given the SNAT2 \( K_m \) for glutamine of 1.8 mM (2). We investigated the possibility that this might result from the onset of trans-inhibition, a rapidly invoked kinetic phenomenon consistently reported for System A whereby a high intracellular concentration of substrate inhibits cellular AA influx through the transporter (4, 15). To assess the likely extent of trans-

![Fig. 5. Intracellular concentrations of neutral AAs in oocytes (expressing System A and/or L as indicated) incubated for 24 h in MBM containing a physiological plasma AA mixture (6). Top: results for selected System A and/or System L substrates. Bottom: sum totals of net changes (after subtraction of control value) for known AA substrates of System A (Ala, Asn, Gly, Ser, Thr, Gln, Pro, Met, His; some of which are also System L substrates) and for selective System L substrates (Leu, Ile, Val, Phe, Tyr, Thr). Bars show mean ± SE values of experiments using 4 different batches of oocytes. LH axis scales the cellular AA concentrations in units of pmol/oocyte; RH axis scales the same data in units of \( \mu \)mol AA/liter [calculated assuming a water space of 0.5 l/oocyte (2)]. Filled bars show AA concentrations in extracellular MBM, scaled to the RH axis. *\( P < 0.05 \) from respective control value; ⋅\( P < 0.05 \) from respective System A value; ⋅\( P < 0.05 \) from respective System L value. No significant effects on concentrations of cationic (Lys, Arg) or anionic (Glu, Asp) AAs were observed in these experiments.](http://ajpendo.physiology.org/doi/10.220.33.5)

![Fig. 6. Effect of changes in intracellular concentrations of glutamine or leucine on AA tracer metabolism (oxidation, protein incorporation) in *Xenopus* oocytes. Oocytes were injected with 50-nl solution containing either L-[\(^{3}\)H]glutamine or L-[\(^{3}\)H]leucine tracers ± unlabeled glutamine or leucine (to give nominal final concentrations of 5 mM unlabeled AA in each oocyte) and allowed 30-min recovery period in MBM to allow healing of the plasma membrane. They were then transferred to fresh MBM (t = 0), and tracer metabolism was measured over the subsequent 2-h (leucine) or 4-h (glutamine) period. Values shown are means ± SE for 3 (glutamine) or 4 (leucine) batches of oocytes. *\( P < 0.05 \) from basal value.](http://ajpendo.physiology.org/doi/10.220.33.5)
inhibition in our system, we injected known quantities of AA into System A-expressing oocytes. Injected MeAIB produced a clear, concentration-dependent trans-inhibition effect, reducing System A activity by up to 60% (Fig. 8) compared with oocytes injected with sucrose as an osmotic loading control. Comparable trans-inhibitory effects were obtained in experiments where oocytes were preloaded by incubation with MeAIB or, to a lesser extent, serine (Fig. 8).

DISCUSSION

The current results show a clear relationship between cooverexpression of System A (SNAT2) and System L (4F2hc/LAT1) transporters in Xenopus oocytes and their ability to influence intracellular AA concentrations by flux-coupling to produce tertiary active transport (5) of System L substrates such as leucine. Notably, cooverexpression of System A produces a substantial increase in intracellular accumulation of both leucine and isoleucine compared with System L alone (to concentrations over five times higher than the extracellular medium; Fig. 5A), despite the fact that these AAs are not System A substrates. Observations that the downstream consequences of pharmacological blockade or suppression of System A activity in mammalian cell lines include reduced cellular levels of System L substrates such as leucine (11, 16), alongside those showing that increased SNAT2 transport activity during volume regulatory increase results in elevations of both System A substrates and nonsubstrates including leucine (7), offer further evidence for the occurrence and physiological relevance of tertiary active AA transport of this type. The changes of intracellular leucine concentration we measured in the present study are of sufficient magnitude to influence the TOR nutrient-signaling pathway in Xenopus oocytes [which is able to detect small (<10%) changes in intracellular leucine concentration (6)]; hence, tertiary active transport represents a mechanism to extend the range of AA stimuli for this pathway, given that AAs such as glutamine are not able to activate TOR directly when injected into oocytes. Expression of both 4F2hc/LAT1 (10, 19) and SNAT2 (11, 16) show positive correlation with TOR pathway activation, and their functional coupling may help explain why in certain circumstances glutamine is required to enable extracellular leucine to activate the TOR pathway (12). Nicklin et al. (23) have recently shown that an AA “shuttle” involving glutamine uptake by the high-affinity SLC1A5 (ASCT2; System ASC) transporter primes leucine uptake through System L in AA-depleted HeLa cells. SNAT2 and ASCT2 are both able to accumulate glutamine within a cell in a Na⁺-dependent manner (17, 20), although SNAT2 is not fully saturated (and therefore likely to be more responsive) at physiological plasma AA concentrations and is not dependent on the presence of intracellular AA [SLC1A5 is a Na⁺-dependent AA exchanger (17)]. An additional mechanism by which leucine and glutamine may combine to activate the TOR pathway is proposed to involve increased mitochondrial metabolism by oxidative decarboxylation of leucine and allosteric activation of glutamate dehydrogenase by glutamine (34).

Another striking conclusion from our studies is that membrane transport appears to be rate limiting for intracellular glutamine- and leucine-consuming processes in native oocytes,
because both AAs are catabolized within the oocyte and their cytoplasmic concentrations are maintained below extracellular levels. Leucine metabolism is stimulated by cytoplasmic glutamine injection, indicating that the increases in cell leucine concentration that we report in Figs. 5 and 7 are not due to reduced leucine catabolism and implying that these data may actually underestimate the potential of tertiary active transport to accumulate leucine in our experimental system. We have attempted to produce a semiquantitative description of the AA fluxes determining the intracellular leucine and glutamine pool sizes in oocytes bathed in AA-containing medium (Fig. 9). This description reveals the continued importance of endogenous AA fluxes in establishment of steady-state AA distributions and helps explain why the intracellular concentrations of System A substrates achieved were relatively modest given the prevailing Na+-electrochemical potential for cosubstrate accumulation (2, 27, 35), being only around 2–5 times higher than those in the external medium. At least for glutamine, this results from the relatively low level of functional overexpression of SNAT2 compared with the basal permeability of the oocyte membrane to glutamine: given a rate constant for glutamine efflux of 0.20 h⁻¹ and glutamine influx of 165 pmol·oocyte⁻¹·h⁻¹ at 0.67 mM glutamine in SNAT2-expressing oocytes (estimated from values reported in Ref. 2), fluxes would balance when intracellular glutamine concentration reached around 800 pmol/oocyte (1.6 mM) as observed (Fig. 5A). Net glutamine uptake through System L is not favored in the physiological AA mix (6, 21), and the effects of coexpressing System L alongside System A on glutamine balance are seen principally as an enhanced net glutamine efflux through System L overlying a reduced endogenous efflux due to the fall in intracellular glutamine concentration (from ~800 to 550 pmol/oocyte). In contrast, the effects of coexpressing System A alongside System L on leucine balance are seen mainly as an increased net leucine uptake through System L (driven by the coupled efflux of Gln, Met, Ser, etc., along their enhanced concentration gradients) overlying increases in endogenous efflux and leucine oxidation.

The model described in Fig. 9 also highlights the interplay between AA fluxes and the coupling between System A and System L transporters. The extent of System L trans-stimulation by intracellular glutamine under conditions relevant to our experimental system (0.5–1 nmol Gln/oocyte) is relatively small, although it is consistent with previous reports (21), in which high levels of trans-stimulation are only observed at higher intracellular [AA] of 2–10 nmol/oocyte and is clearly sufficient to mediate the overall net increase of intracellular leucine over the 4- to 12-h experimental periods. The fact that the reciprocal changes in System A and System L substrate concentrations that we observed do not match exactly, as would be expected if driven solely by a 1:1 heteroexchange mechanism, might be in part explained by the presence of unstirred aqueous layers near the oocyte membrane as well as by a significant contribution of metabolism to AA distribution. Of the AAs recognized as both Systems A and L substrates in our physiological AA mixture, glutamine (the most abundant) and methionine [the most potent trans-stimulator (21)] appear to be particularly important contributors to the tertiary active transport process (see Fig. 5A); serine, threonine, and histidine also made net contributions. Leucine, isoleucine, and valine accounted for most of the net increase in System L substrates. The importance of providing a range of possible substrates for heteroexchange to effectively preload or “prime” cells for leucine uptake is illustrated by the observations that 1) 5 mM leucine alone was much less effective than the AA mix in terms of increasing cell leucine concentration in System L-expressing oocytes (at least over a 4-h period), and 2) the reciprocal changes in glutamine and leucine concentration in Gln→Leu loading experiments (during which Gln-Gln or Leu-Leu homoechangexes would tend to predominate) were also lesser in magnitude.

Trans-inhibition of SNAT2 activity may help explain why oocytes incubated in 5 mM glutamine show no greater accumulation of this AA than do those in a physiological mixture containing 0.67 mM Gln. Addition of the physiological AA mix does not significantly depolarize oocytes (6); hence, a major influence of membrane potential on AA-dependent mod-

---

**Fig. 9.** Semiquantitative estimates of glutamine and leucine fluxes contributing to steady-state cytoplasmic free-AA pools of experimental oocytes cultured in MBM containing a physiological mixture of AA (including 0.67 mM Gln and 0.16 mM Leu). Values were calculated on basis of radiotracer flux measurements, and AA concentrations are presented in Figs. 2–7 and published data for selectivity and relative AA Km values for Systems A (2, 20, 35) and L (21, 26, 31), assuming that proteolysis (estimated as the sum of AA oxidation and efflux from control oocytes in MBM) was constant. Appearance/disappearance represent the sum of all AA flows entering/leaving (respectively) the intracellular free-AA pool; thus, influx across the plasma membrane contributes to appearance and efflux to disappearance. Estimated value for glutamine appearance by proteolysis may include a component of endogenous glutamine synthesis. Endogenous AA flux values are based on data from control oocytes, assuming the absence of trans-effects; rate constants for endogenous efflux of glutamine and leucine averaged 0.20 and 0.35 h⁻¹, respectively, for control oocytes in MBM.
ulation of transport is unlikely. Glutamine and MeAIB are the most potent trans-inhibitory AA substrates of System A (4, 15), which may explain the predominant effect of glutamine observed here. Our results are consistent with the view that trans-inhibition may involve “trapping” of SNAT2 in an inwardly facing direction by binding of cytoplasmic AA, which prevents the conformational change necessary for the unloaded transporter to complete the transport cycle by returning to the extracellular face of the membrane (4, 15). The effect appears to be competitive, suggesting a single binding site involved in both cis- and trans-inhibition. Trans-inhibition will tend to limit the cell swelling and toxicity associated with overaccumulation of AAs (4, 7, 15) and may represent an underappreciated mechanism for regulation of the intracellular AA pool size (particularly in cells lacking other Na+-dependent AA transporters) (25). Trans-inhibition has also been reported for the bile salt export pump (30), rOCT2 (33), and astrocyte glutamate transporter (8).

In summary, we have shown that sequential coupling of neutral amino acid fluxes through concentrative and dissipative transporters (Systems A and L, respectively) may, by tertiary active transport, enhance the ability of cells to accumulate amino acids such as leucine and, hence, promote cellular anabolism. This amino acid flux-coupling is enabled by the maintenance (or rapid initial development) of an outwardly directed concentration gradient of a group of amino acids, exemplified by glutamine, which are substrates of both transporter types. There are limitations to directly extrapolating these studies in Xenopus oocytes to mammalian cells, but the present observations may substantially underestimate the likely effect of tertiary active transport in smaller mammalian cells, where transcellular amino acid gradients tend to be higher (see Ref. 5 for review). The overall effects in vivo will also depend on the relative activity of concentrative and dissipative transporters, the metabolic profile of specific tissues, and the dynamics of plasma amino acid concentrations (e.g., during periodic feeding cycles). System A is the principal insulin-regulated amino acid transporter in mammalian cells, and thus it is plausible that insulin stimulation of System A activity may contribute indirectly to the nutrient-induced activation of mTOR via tertiary active transport. If so, this may prove to be important for stimulating protein synthesis both by modulating the phosphorylation/activity status of proteins involved in mRNA translation and by increasing provision of amino acids to the free pool to support high levels of the process. Amino acid flux-coupling of this type may also help amplify the anabolic signal during lymphocyte activation, where cell growth is closely associated with enhanced expression of System L transporter subunits (24).

ACKNOWLEDGMENTS

We are very grateful to Dr. J. D. Erickson (LSU, New Orleans, LA) for providing the pTLN2-rat SNAT2 DNA and Dr Yun-Bo Shi (National Institute of Child Health and Human Development, Bethesda, MD) for the IU12 cDNA. We also thank Fraser Simpson, Jane Halcrow, William Ogilvie, Sophia Pardakis, and Thomas Maden-Wilkinson for technical assistance.

GRANTS

This work was supported by the UK Medical Research Council, UK Biotechnology and Biological Sciences Research Council, Tenovus Tayside, and the University of Dundee.

REFERENCES


Downloaded from http://ajpendo.physiology.org/ by 10.220.33.5 on November 7, 2017


