Amino Acids (2012) 43:2561–2567
DOI 10.1007/s00726-012-1312-0

SHORT COMMUNICATION

Glutamine stimulates mTORC1 independent of the cell content of essential amino acids

Martina Chiu · Saverio Tardito · Amelia Barilli · Massimiliano G. Bianchi · Valeria Dall'Asta · Ovidio Bussolati

Received: 23 February 2012 / Accepted: 20 April 2012 / Published online: 8 May 2012
© Springer-Verlag 2012

Abstract Glutamine and leucine are important mTORC1 modulators, although their roles are not precisely defined. In HepG2 and HeLa cells glutamine-free incubation lowers mTORC1 activity, although cell leucine is not decreased. mTORC1 activity, suppressed by amino acid-free incubation, is completely rescued only if essential amino acids (EAA) and glutamine are simultaneously restored, although cell leucine is higher in the absence than in the presence of glutamine. Thus, glutamine stimulates mTORC1 independent of cell leucine, suggesting the existence of two distinct stimulatory signals from either glutamine or EAA.

Keywords Glutamine · Essential amino acids · Leucine · mTOR · S6K1

Introduction

The kinase mTOR, an important control point of cell metabolism, operates through two complexes, mTORC1 and mTORC2, with different molecular partners, substrates, and biological activities (Sengupta et al. 2010; Zoncu et al. 2011). mTORC1 activity, which accounts for mTOR-dependent effects on protein synthesis and autophagy, is simultaneously controlled by the presence of growth factors, the energy status of the cell, the availability of amino acids, and the subsistence of stress conditions of different origin (Sengupta et al. 2010). The relative importance of these factors, as well as the precise mechanism through which each of them affects mTORC1 activity, is still matter of debate.

As far as amino acids are concerned, it has been known since many years that mTORC1 is sensitive to the availability of essential amino acids (EAA), such as, in particular, leucine (Fox et al. 1998; Kimball et al. 1999). Leucine affects mTORC1 activity through a pathway that, involving the GTPases RAGs, allows the interaction of mTOR, with the activator protein Rheb promoting the localization of the kinase in a specific intracellular compartment (Sancak et al. 2008; Avruch et al. 2009). Most recently, leucyl-tRNA synthetase has been specifically involved in the RAG-dependent activation of mTORC1 by leucine and other EAA (Han et al. 2012). However, also some non-essential amino acids, such as glutamine (Gleason et al. 2007) and arginine (Bauchart-Thevret et al. 2010), heavily influence mTORC1 activity. In particular, due to its central role in nitrogen metabolism, the relationship between glutamine availability and leucine-dependent mTORC1 stimulation has been repeatedly investigated, producing somewhat conflicting results. Indeed, while in murine myogenic C2C12 cells (Deldicque et al. 2008) and in rat intestinal cells (Nakajo et al. 2005), glutamine has been indicated as an inhibitor of leucine-dependent mTORC1 activation, in other models it has been described as a co-stimulator of the kinase (Xu et al. 2001; Krause et al. 2002; Xia et al. 2003).

M. Chiu and S. Tardito contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s00726-012-1312-0) contains supplementary material, which is available to authorized users.

M. Chiu · S. Tardito · A. Barilli · M. G. Bianchi · V. Dall'Asta · O. Bussolati (✉)
Unit of General and Clinical Pathology, Department of Experimental Medicine, University of Parma, Via Volturno 39, 43125 Parma, Italy
e-mail: ovidio.bussolati@unipr.it

M. Chiu
Doctorate School of Molecular Medicine, University of Milan, Milan, Italy

However, these studies have not elucidated the pathway through which glutamine affects mTORC1 activity. Given that glutamine availability markedly influences cell volume (Dall'Asta et al. 1999), changes in cell volume have been implied in the glutamine effects on mTOR (Hundal and Taylor 2009). Studies in muscle cells have attributed to the SNAT2 transporter, a secondary active mechanism that contributes to the uphill transport of the amino acid, an important role in glutamine-dependent mTOR stimulation (Evans et al. 2007, 2008). More recently, the hypothesis that glutamine controls mTORC1 only indirectly, i.e. producing changes in the intracellular levels of leucine, has been advanced using HeLa cells (Nicklin et al. 2009). According to this view, intracellular glutamine, interacting with exchange transport systems for amino acids, such as ASCT2, may fuel the entry of leucine that, subsequently, would be responsible for mTORC1 stimulation.

To investigate further these issues, we have studied the interaction between glutamine and EAA in mTORC1 stimulation in two different cell models, HepG2 and HeLa cells, demonstrating that glutamine is able to influence mTORC1 activity without changing the cell content of leucine and that mTORC1 sensitivity to amino acids may have cell type specific traits.

Materials and methods

Cell lines, experimental treatments and viability

HepG2 cells (a gift by Prof. Giovanni Raimondo, Department of Internal Medicine, University of Messina, Italy) were grown in low-glucose (1 g/l) Dulbecco's modified Eagle's medium (DMEM, cat. n. ECM0749L, EuroClone) containing 1 mM sodium pyruvate. HeLa cells, obtained from ATCC, were maintained in high-glucose (4.5 g/l) Dulbecco's modified Eagle's medium (DMEM, cat. n. ECB7501L, EuroClone), enriched with 1 mM sodium pyruvate. For both cell cultures, the medium was supplemented with 10 % fetal bovine serum (FBS, Lonza, Basel, Switzerland), 4 mM glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin, and maintained at 37 °C in an atmosphere of 5 % CO_2 in air, pH 7.4. After thawing, the cultures were not used for more than nine passages.

For the experiments, cells were seeded in complete growth medium in 6-well plates (Falcon, 1×10^6 HepG2, 0.5×10^6 HeLa) and grown for 24 h. Cells were then washed with Earle's Balanced Salt Solution (EBSS, composition in mM: NaCl 116, KCl 5.3, $CaCl_2$ 1.8, $MgSO_4 \cdot 7H_2O$ 0.81, $NaH_2PO_4 \cdot H\ _2O$ 0.9, $NaHCO_3$ 26, glucose 5.5, supplemented with 0.02 % Phenol Red) and incubated in DMEM with or without glutamine, supplemented with dialyzed serum. Treatment periods and glutamine concentrations are detailed for each experiment. For the experiments shown in Fig. 2, cells were washed and incubated for 3 h in EBSS without FBS. Starved cells were then incubated for 1 h with glutamine (4 mM) and/or EAA from the MEM EAA solution (Invitrogen cat. No. 11130-036).

Western blot

Cells were lysed in a buffer containing 20 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 % Triton, 2.5 mM sodium pyrophosphate, 1 mM *β*-glycerophosphate, 1 mM Na_3VO_4, 1 mM NaF, 2 mM imidazole and a cocktail of protease inhibitors (Complete, Mini, EDTA-free, Roche). Lysates were transferred in Eppendorf tubes, sonicated for 5 s and centrifuged at 12,000*g* for 10 min at 4 °C. After quantification with the Bio-Rad protein assay, aliquots of 25 μg of proteins were mixed with Laemmli buffer 4× (250 mM Tris–HCl, pH 6.8, 8 % SDS, 40 % glycerol, and 0.4 M DTT), warmed at 95 °C for 5 min and loaded on a 10 % gel for SDS-PAGE. After electrophoresis, proteins were transferred to PVDF membranes (Immobilon-P, Millipore, Millipore Corporation, MA, USA). Non-specific binding sites were blocked with an incubation of 2 h at room temperature in 5 % bovine serum albumin (BSA) in TBS-Tween solution. The blots were then exposed at 4 °C overnight to the following antibodies diluted in a 5 % BSA TBS-Tween solution: anti-total p70S6K or anti-phospho-p70S6K (T389 or T421-S424, rabbit monoclonal, 1:1,000, Cell Signaling Technology); anti-phospho-4E-BP1(S65, rabbit polyclonal, 1:1,000, Cell Signaling Technology); glutamine synthetase (GS, mouse monoclonal, 1:2,500, BD Transduction Laboratories); *β*-tubulin (mouse monoclonal, 1:2,000, Santa Cruz Biotechnology). After washing, the blots were exposed for 1 h at room temperature to HRP-conjugated anti-mouse or anti-rabbit antibody (Cell Signaling Technology), diluted 1:10,000 in blocking solution. Immunoreactivity was visualized with Immobilon Western Chemiluminescent HRP Substrate (Millipore).

Cell content of amino acids

The intracellular content of amino acids was determined as previously described (Dall'Asta et al. 1999) with minor modifications. Briefly, cell monolayers were washed twice with ice-cold PBS, extracted in a solution of acetic acid (6.6 mM) in ethanol and processed for protein determination with the Lowry method. After lyophilization and reconstitution in LiOH buffer (pH 2.2), the intracellular content of the single amino acids was determined by HPLC analysis with a Biochrom 20 amino acid analyzer (Amersham Pharmacia Biotech), employing a high-resolution

column (Bio 20 Peek Lithium) and the physiological fluid chemical kit (Amersham Pharmacia Biotech) for elution. The column effluent was mixed with ninhydrin reagent, passed through the high-temperature reaction coil, and read by the photometer unit. Data are expressed as nmol/mg of protein.

Statistics

For statistical analysis two-tailed Student's *t* test for unpaired samples was used.

Materials

Sigma (Milan, Italy) was the source of all the chemicals, whenever not indicated otherwise.

Results

HepG2 cells, incubated for 24 h at different concentrations of Gln, exhibited a Gln-dependent decrease of the phosphorylation of p70S6K1 at T389-T412, an exclusive target of mTORC1, as well as of T421/S424 and T444/S447, phosphorylation sites regulated by both mTORC1 and MAP kinases (Fig. 1a). However, even in cells incubated in the complete absence of Gln, the phosphorylated form of S6K1 remained clearly detectable, indicating that mTORC1 activity was not completely suppressed. Gln depletion was also associated with a progressive increase in the abundance of glutamine synthetase (GS). 4E-BP1, another mTORC1 substrate involved in the regulation of protein synthesis (Ma and Blenis 2009) was also sensitive to extracellular Gln, although phospho-4E-BP1 decreased less markedly than phospho-p70S6K1 (Supplementary Fig. 1). The comparison of the intracellular pool of amino acids of HepG2 cells, maintained at 4 mM Gln or incubated for 24 h in the absence of the amino acid (Fig. 1b), indicated that Gln starvation produced a marked decrease in the cell contents of Glu and Ala, besides the almost complete depletion of cell Gln. On the contrary, the cell content of the EAA Thr, Ile, Leu, Tyr, Phe, and His was higher in the absence than in the presence of 4 mM extracellular Gln.

The time course of changes in mTORC1 activity during Gln depletion of HepG2 cells (Fig. 1c) indicated that the activity of the kinase was almost abolished from 3 to 16 h of treatment, when the levels of phospho-T389-T412 p70S6K1 were barely detectable. However, phospho-p70S6K1 increased significantly at longer times of Gln depletion, although it remained lower than those detected under control conditions (extracellular [Gln] = 4 mM). Also in HeLa cells (Fig. 1d), glutamine depletion caused a biphasic modification of mTORC1 activity, with a dramatic change detected at 6 h of depletion, and a marked, although partial, restoration of phospho-p70S6K1 levels at later times. The intracellular content of amino acids was determined in parallel cultures at the same experimental times in which kinase activity was tested (Fig. 1e, f, Supplementary Fig. 2). In both HepG2 and HeLa cells, at short times of Gln-free incubation, intracellular Gln almost disappeared and the cell content of glutamate markedly decreased, while cell leucine was not markedly changed. After prolonged incubation in Gln-free medium, cell glutamate remained substantially unchanged, while leucine content progressively increased to reach a level higher than that determined in control cultures, always maintained at 4 mM Gln. Leucine behavior was shared by the other essential amino acids.

A short-term (3 h) incubation of HepG2 cells in unsupplemented saline solution (EBSS) led to the disappearance of phospho-S6K1 (Fig. 2a). Consistently, phospho-4E-BP1 also became undetectable under the same conditions (data not shown), indicating that mTORC1 activity was completely suppressed. The restitution of extracellular Gln to starved cells (4 mM for 1 h) did not cause any increase in phospho-S6K1 (Fig. 2a), which, conversely, became evident upon the restitution of both Gln and EAA at the concentration normally present in the growth medium (EAA 1×). Under the same condition, phospho-4E-BP1 also became clearly evident (data not shown). In the absence of Gln, EAA were also unable to restore mTORC1 activity, even when added at extracellular concentrations two- (2×) or three-fold higher (3×) than those present in standard growth medium. Under the same conditions, also phospho-4E-BP1 was only partially marginally activated by restitution of Gln alone. Also in HeLa cells phospho-S6K1 was not detectable after amino acid starvation (Fig. 2b) but, at variance with what observed in HepG2 cells, the restitution of EAA (without Gln) or of Gln alone was able to stimulate mTORC1 activity. However, the expression of phospho-S6K1, detected in either case, was much lower than that observed upon the simultaneous restitution of Gln and EAA. .

The analysis of the intracellular amino acid pool (Fig. 2c, d, Supplementary Fig. 3) indicated that in amino acid starved HepG2 cells, incubated for 1 h with EAA (1×), the content of essential amino acids was substantially comparable in the presence and in the absence of extracellular Gln (Fig. 2c). When EAA were added at 2× or 3× concentrations, their intracellular levels were always markedly higher than those obtained upon the simultaneous incubation with EAA 1× and Gln. Comparable results were obtained with HeLa cells (Fig. 2d), where the cell content of leucine, measured 1 h after the restitution of the extracellular amino acid, was markedly higher in the absence than in the presence of Gln.

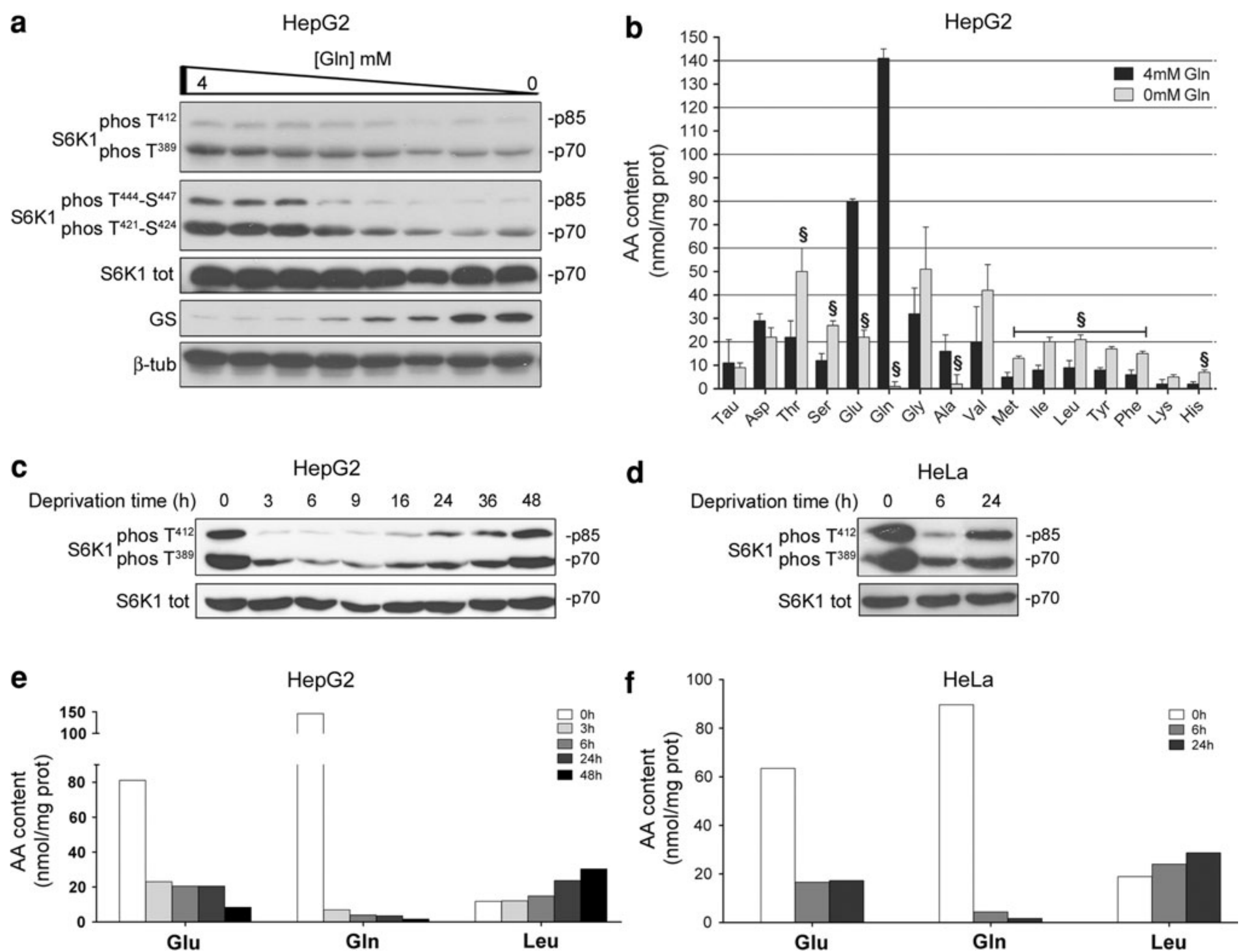
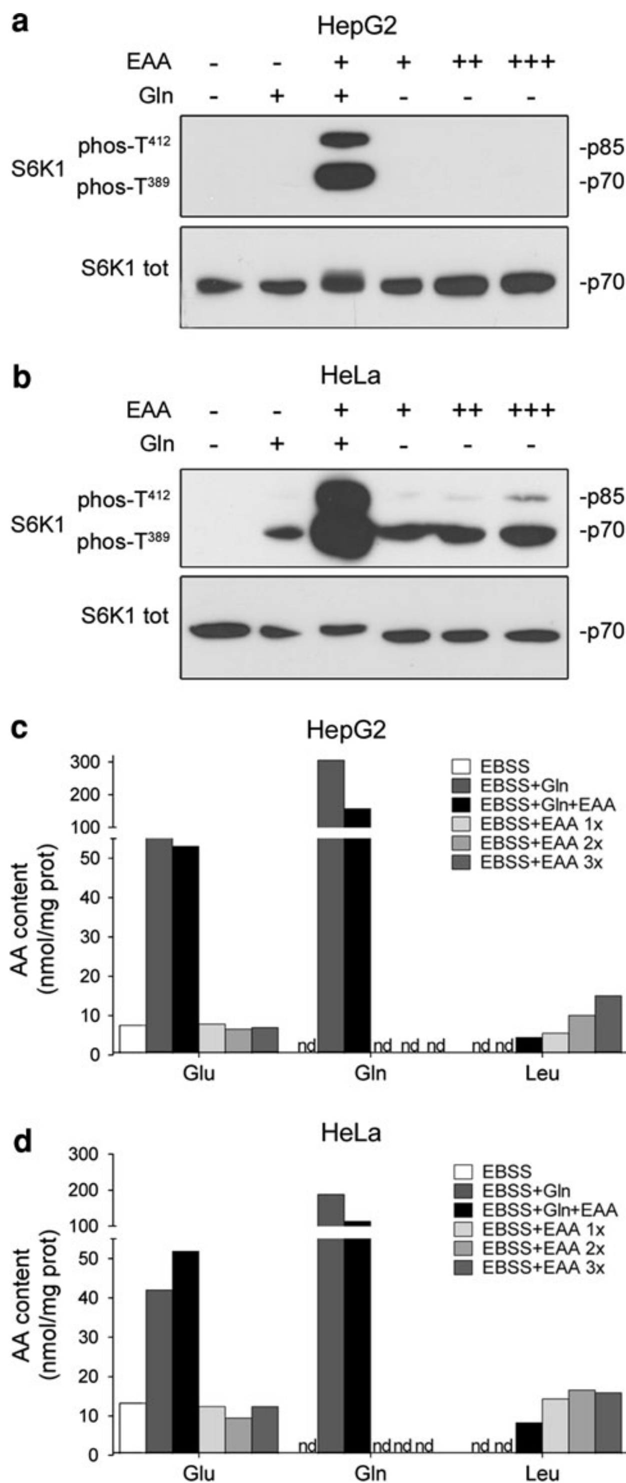


Fig. 1 mTORC1 activity and amino acid content of HepG2 and HeLa cells incubated at different Gln concentrations. HepG2 cells were grown for 24 h in complete growth medium, supplemented with 10 % FBS. Incubation medium was then replaced by DMEM supplemented with 10 % dialyzed FBS and the following concentrations of Gln (in mM): 4, 2.25, 1.26, 0.71, 0.4, 0.22, 0.125, 0. **a** After 24 h of incubation at the indicated Gln concentrations, the expression of phospho- and total S6K1 kinase and of Glutamine Synthetase were assessed with western blot. β-Tubulin was used as loading control. The experiment was repeated twice with comparable results. **b** The intracellular amino acid pool of HepG2 cultures incubated in the absence or in the presence of Gln (4 mM) for 24 h was determined as described in "Materials and methods". Data are means of three independent experiments with SD shown. § $p < 0.05$ versus control at 4 mM glutamine. **c, d** HepG2 (**c**) and HeLa cells (**d**) were grown for 24 h in complete growth medium, supplemented with 10 % FBS. Incubation medium was then replaced by Gln-free DMEM, supplemented with 10% dialyzed FBS, for the indicated times. The expression of total and phosphorylated S6K1 kinase was determined with Western blot analysis. **e, f** In parallel, the intracellular amino acid content was determined in HepG2 (**e**) and HeLa cells (**f**), as described in "Materials and methods". See Supplementary Fig. 2 for the complete amino acid pool. The results of a representative experiment, performed twice, are shown

Discussion

Recent studies have identified some components of the amino acid sensing machinery that controls mTORC1 activity, such as RAG proteins (Sancak and Sabatini 2009), leucyl-tRNA Synthetase (Han et al. 2012), PAT transporters (Goberdhan 2010), or the transporter SNAT2 (Hundal and Taylor 2009). However, with the possible exception of leucyl-tRNA Synthetase, what these components sense and how they transmit the signals to mTOR complex are still incompletely defined (Wang and Proud 2011). In particular, although it is known that some amino acids, such as leucine and glutamine, are particularly important for mTORC1 regulation, it is not known if they act collectively or independent of each other. Recently, it has been proposed that, in HeLa cells, Gln stimulates mTORC1 increasing the intracellular availability of leucine through a transport exchange mechanism (Nicklin et al. 2009). Moreover, contrasting literature evidence points either to a stimulatory (Xu et al. 2001; Krause et al. 2002; Xia et al. 2003) or to an inhibitory (Nakajo et al. 2005; Deldicque et al. 2008) role of Gln on mTORC1 activity. In



◀ **Fig. 2** Effect of restitution of extracellular amino acids on mTORC1 activity of starved cells. Growth medium of HepG2 (**a, c**) or HeLa cells (**b, d**) was replaced by EBSS in the absence of FBS. After 3 h the incubation medium was replaced by fresh EBSS or by EBSS supplemented with 4 mM Gln and/or Essential Amino Acids (EAA 1×, +, at the concentration present in MEM, see "Materials and methods") or at doubled (2×, ++) or tripled (3×, +++) concentration, as indicated. The incubation was prolonged for 1 h. **a, b** Cells were then lysed and the expression of total and phosphorylated S6K1 kinase was assessed with western blot. **c, d** At the end of the experiment the intracellular amino acid content was determined, as described in "Materials and methods", in parallel cultures. See Supplementary Fig. 3 for the complete amino acid pool. The experiments were performed twice with comparable results

most of these contributions, intracellular amino acid levels were not directly measured under the different experimental conditions and, in particular, under Gln-free conditions.

In this contribution, we measure in parallel the intracellular amino acids and mTORC1 activity and show that, in human hepatocellular carcinoma HepG2 cells, Gln is able to modulate mTORC1 independent of changes in the cell content of leucine (or of other EAA). Indeed, when HepG2 cells are incubated in complete growth medium in the absence of Gln, mTORC1 activity is severely depressed, although the cell content of EAA, and in particular of leucine, is not decreased. Moreover, in the same cell model, the attainment of a threshold level of intracellular leucine does not appear to be the sole factor that regulates mTORC1 activity. Indeed, after a severe depletion of cell amino acids, obtained through an incubation in plain saline solution, the restitution of extracellular EAA, even at supra-physiological concentrations, is not able to rescue mTORC1 activity. Interestingly, under such conditions, the cell content of leucine (and of the other EAA) is markedly higher than that determined under control conditions, when mTORC1 activity is high. However, also the supplementation of saline solution solely with glutamine is not sufficient to rescue mTORC1 activity, a result consistent with previous reports (Wen et al. 2005; Nicklin et al. 2009).

Gln depletion yielded similar results in HeLa cells. Also in this cell model Gln-free incubation suppresses mTORC1 activity, without lowering the cell content of leucine and of other EAA. When mTORC1 activity is abolished by amino acid-free incubation, the simple restitution of EAA or Gln triggers only a partial rescue of mTORC1 activity that, as in HepG2 cells, is completely achieved only when both EAA and Gln are concomitantly supplied. This synergistic stimulation of mTORC1 is not due to the increase in the intracellular content of leucine and Gln, since they are accumulated into the cells at higher levels when added separately rather than together (Fig. 2d).

The simplest explanation for these observations is that both leucine and glutamine must be present to produce the maximal activation of mTORC1. If this is true, the signals and transduction pathways involved may be also distinct and their sensitivities different. Alternatively, leucine influx, rather than its concentration inside the cell, may be important for mTORC1 activation. In this case, Gln accumulation, mediated by concentrative transport systems, such as ATB0+ or SNAT2, may play the fundamental role

of promoting EAA influx through exchange mechanisms, such as LAT1 and ASCT2. We have recently demonstrated that the non-proteinogenic amino acids L-methionine sulfoximine and DL-phosphinothricin, endowed with structural resemblance with Gln, can also powerfully synergize EAA in mTORC1 stimulation when Gln is absent (Tardito et al. 2011a), indicating that they behave as Gln analogues in that experimental context. These compounds may prove useful tools to dissect mTORC1 activation pathway and, in particular, to verify if they are able, once accumulated into the cell, to stimulate leucine influx.

Glutamine depletion does not only affects mTORC1, but also the expression and the activity of other enzymes, such as glutamine synthetase (GS), which accounts for the intracellular synthesis of glutamine. It has been known since many years that in a variety of cell models glutamine depletion leads to an increase in GS protein shelf life and, therefore, to an adaptive, transcription-independent increase in GS activity (Feng et al. 1990; Labow et al. 1998; Labow et al. 1999; Rotoli et al. 2005; Tardito et al. 2011b). Results reported in Figs. 1 and 2 indicate that, if cells are incubated in the absence of Gln, the intracellular content of leucine (as well as that of other EAA) is higher than in the presence of 4 mM extracellular Gln. This finding is clearly counterintuitive, since it is well known that, through accumulation through secondary active transport systems and successive efflux mediated by exchange routes, Gln can effectively energize the influx of leucine and of the other EAA. This “tertiary” active transport was proposed several years ago by our laboratory (Bussolati et al. 2001), successively demonstrated experimentally by others (Baird et al. 2009) and eventually applied to explain mTORC1 regulation by Gln (Nicklin et al. 2009). Yet, the inhibitory effect of (high) extracellular glutamine on EAA cell levels has been consistently found in our experiments, both in HepG2 and HeLa cells, suggesting that the transport interaction between Gln and EAA may be more complex than previously thought. In particular, it is possible that the effect of the tertiary active transport is counterbalanced, and even overcome, by direct competition of Gln and EAA for a shared, secondary active transport system, endowed with a sufficiently broad specificity. A candidate for this role may be ATB0+, which is actually the only transporter, able to accumulate all the amino acids, that are known stimulators of mTORC1 (Karunakaran et al. 2011). Interestingly, the mRNA of *SLC6A14*, the gene that encodes for ATB0+, is threefold increased in Gln-depleted HepG2 cells (unpublished results).

The time course of mTORC1 activity during Gln-free incubation indicates that its pattern is biphasic, with a minimum at earlier times of incubation (3 h and 6 h) and a subsequent, partial rescue at later times. The restoration of kinase activity is heralded by the increase of glutamine synthetase levels, already evident after 3 h of depletion, and by the appearance of the autophagic marker LC3(II) at 9 h and 16 h of depletion (data not shown). It is, therefore, tentative to attribute the rescue of mTORC1 activity to these adaptive mechanisms, both able to increase intracellular amino acid availability.

In summary, the main conclusions reached in this study are that (a) Gln influences mTORC1 activity independent of its effects on the cell content of leucine and that (b) both Gln and leucine appear necessary for the maximal stimulation of mTORC1, although the sensitivity of the kinase activity to their effect may vary in different cell models. Moreover, we demonstrate that, under certain conditions, the stimulatory effect of Gln on mTORC1 is not explained by the stimulation of leucine accumulation by cell glutamine. Thus, we propose that Gln and leucine activate mTORC1 activity through distinct and synergistic mechanisms.

Acknowledgments M.C. is supported by a fellowship of the Doctorate School of Molecular Medicine, University of Milan. A.B. and M.G.B. are supported by research scholarships of the University of Parma Medical School.

Conflict of interest The authors declare that they have no conflict of interest.

References

Avruch J, Long X, Ortiz-Vega S, Rapley J, Papageorgiou A, Dai N (2009) Amino acid regulation of tor complex 1. Am J Physiol Endocrinol Metab 296:E592–E602

Baird FE, Bett KJ, MacLean C, Tee AR, Hundal HS, Taylor PM (2009) 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

Bauchart-Thevret C, Cui L, Wu G, Burrin DG (2010) Arginine-induced stimulation of protein synthesis and survival in ipec-j2 cells is mediated by mtor but not nitric oxide. Am J Physiol Endocrinol Metab 299:E899–E909

Bussolati O, Dall’Asta V, Franchi-Gazzola R, Sala R, Rotoli BM, Visigalli R, Casado J, Lopez-Fontanals M, Pastor-Anglada M, Gazzola GC (2001) The role of system a for neutral amino acid transport in the regulation of cell volume. Mol Membr Biol 18:27–38

Dall’Asta V, Bussolati O, Sala R, Parolari A, Alamanni F, Biglioli P, Gazzola GC (1999) Amino acids are compatible osmolytes for volume recovery after hypertonic shrinkage in vascular endothelial cells. Am J Physiol 276:C865–C872

Deldicque L, Sanchez Canedo C, Horman S, De Potter I, Bertrand L, Hue L, Francaux M (2008) Antagonistic effects of leucine and glutamine on the mtor pathway in myogenic c2c12 cells. Amino Acids 35:147–155

Evans K, Nasim Z, Brown J, Butler H, Kauser S, Varoqui H, Erickson JD, Herbert TP, Bevington A (2007) Acidosis-sensing glutamine pump snat2 determines amino acid levels and mammalian target

of rapamycin signalling to protein synthesis in l6 muscle cells. J Am Soc Nephrol 18:1426–1436

Evans K, Nasim Z, Brown J, Clapp E, Amin A, Yang B, Herbert TP, Bevington A (2008) Inhibition of snat2 by metabolic acidosis enhances proteolysis in skeletal muscle. J Am Soc Nephrol 19:2119–2129

Feng B, Shiber SK, Max SR (1990) Glutamine regulates glutamine synthetase expression in skeletal muscle cells in culture. J Cell Physiol 145:376–380

Fox HL, Pham PT, Kimball SR, Jefferson LS, Lynch CJ (1998) Amino acid effects on translational repressor 4e-bp1 are mediated primarily by L-leucine in isolated adipocytes. Am J Physiol 275:C1232–C1238

Gleason CE, Lu D, Witters LA, Newgard CB, Birnbaum MJ (2007) The role of ampk and mtor in nutrient sensing in pancreatic beta-cells. J Biol Chem 282:10341–10351

Goberdhan DC (2010) Intracellular amino acid sensing and mtorc1-regulated growth: new ways to block an old target? Curr Opin Investig Drugs 11:1360–1367

Han JM, Jeong SJ, Park MC, Kim G, Kwon NH, Kim HK, Ha SH, Ryu SH, Kim S (2012) Leucyl-trna synthetase is an intracellular leucine sensor for the mtorc1-signaling pathway. Cell. doi: 10.1016/j.cell.2012.02.044

Hundal HS, Taylor PM (2009) Amino acid transceptors: gate keepers of nutrient exchange and regulators of nutrient signaling. Am J Physiol Endocrinol Metab 296:E603–E613

Karunakaran S, Ramachandran S, Coothankandaswamy V, Elangovan S, Babu E, Periyasamy-Thandavan S, Gurav A, Gnanaprakasam JP, Singh N, Schoenlein PV, Prasad PD, Thangaraju M, Ganapathy V (2011) Slc6a14 (atb0,+) protein, a highly concentrative and broad specific amino acid transporter, is a novel and effective drug target for treatment of estrogen receptor-positive breast cancer. J Biol Chem 286:31830–31838

Kimball SR, Shantz LM, Horetsky RL, Jefferson LS (1999) Leucine regulates translation of specific mrnas in l6 myoblasts through mtor-mediated changes in availability of eif4e and phosphorylation of ribosomal protein s6. J Biol Chem 274:11647–11652

Krause U, Bertrand L, Maisin L, Rosa M, Hue L (2002) Signalling pathways and combinatory effects of insulin and amino acids in isolated rat hepatocytes. Eur J Biochem 269:3742–3750

Labow BI, Abcouwer SF, Lin CM, Souba WW (1998) Glutamine synthetase expression in rat lung is regulated by protein stability. Am J Physiol 275:L877–L886

Labow BI, Souba WW, Abcouwer SF (1999) Glutamine synthetase expression in muscle is regulated by transcriptional and posttranscriptional mechanisms. Am J Physiol 276:E1136–E1145

Ma XM, Blenis J (2009) Molecular mechanisms of mtor-mediated translational control. Nat Rev Mol Cell Biol 10:307–318

Nakajo T, Yamatsuji T, Ban H, Shigemitsu K, Haisa M, Motoki T, Noma K, Nobuhisa T, Matsuoka J, Gunduz M, Yonezawa K, Tanaka N, Naomoto Y (2005) Glutamine is a key regulator for amino acid-controlled cell growth through the mtor signaling pathway in rat intestinal epithelial cells. Biochem Biophys Res Commun 326:174–180

Nicklin P, Bergman P, Zhang B, Triantafellow E, Wang H, Nyfeler B, Yang H, Hild M, Kung C, Wilson C, Myer VE, MacKeigan JP, Porter JA, Wang YK, Cantley LC, Finan PM, Murphy LO (2009) Bidirectional transport of amino acids regulates mtor and autophagy. Cell 136:521–534

Rotoli BM, Uggeri J, Dall'Asta V, Visigalli R, Barilli A, Gatti R, Orlandini G, Gazzola GC, Bussolati O (2005) Inhibition of glutamine synthetase triggers apoptosis in asparaginase-resistant cells. Cell Physiol Biochem 15:281–292

Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L, Sabatini DM (2008) The rag gtpases bind raptor and mediate amino acid signaling to mtorc1. Science 320:1496–1501

Sancak Y, Sabatini DM (2009) Rag proteins regulate amino-acid-induced mtorc1 signalling. Biochem Soc Trans 37:289–290

Sengupta S, Peterson TR, Sabatini DM (2010) Regulation of the mtor complex 1 pathway by nutrients, growth factors, and stress. Mol Cell 40:310–322

Tardito S, Chiu M, Franchi-Gazzola R, Dall'asta V, Comi P, Bussolati O (2011a) The non-proteinogenic amino acids l: methionine sulfoximine and dl: phosphinothricin activate mtor. Amino Acids. doi:10.1007/s00726-011-0981-4

Tardito S, Chiu M, Uggeri J, Zerbini A, Da Ros F, Dall'asta V, Missale G, Bussolati O (2011b) L-asparaginase and inhibitors of glutamine synthetase disclose glutamine addiction of beta-catenin-mutated human hepatocellular carcinoma cells. Curr Cancer Drug Targets 11:929–943

Wang X, Proud CG (2011) Mtorc1 signaling: What we still don't know. J Mol Cell Biol 3:206–220

Wen HY, Abbasi S, Kellems RE, Xia Y (2005) Mtor: A placental growth signaling sensor. Placenta 26 Suppl A, S63–69

Xia Y, Wen HY, Young ME, Guthrie PH, Taegtmeyer H, Kellems RE (2003) Mammalian target of rapamycin and protein kinase a signaling mediate the cardiac transcriptional response to glutamine. J Biol Chem 278:13143–13150

Xu G, Kwon G, Cruz WS, Marshall CA, McDaniel ML (2001) Metabolic regulation by leucine of translation initiation through the mtor-signaling pathway by pancreatic beta-cells. Diabetes 50:353–360

Zoncu R, Efeyan A, Sabatini DM (2011) Mtor: From growth signal integration to cancer, diabetes and ageing. Nat Rev Mol Cell Biol 12:21–35