Intracellular Sensing of Amino Acids in *Xenopus laevis* Oocytes Stimulates p70 S6 Kinase in a Target of Rapamycin-dependent Manner*

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Amino acids exert modulatory effects on proteins involved in control of mRNA translation in animal cells through the target of rapamycin (TOR) signaling pathway. Here we use oocytes of *Xenopus laevis* to investigate mechanisms by which amino acids are “sensed” in animal cells. Small (–48%) but physiologically relevant increases in intracellular but not extracellular total amino acid concentration (or Leu or Trp but not Ala, Glu, or Gln alone) resulted in increased phosphorylation of p70^S6K^ and its substrate ribosomal protein S6. This response was inhibited by rapamycin, demonstrating that the effects require the TOR pathway. Alcohols of active amino acids substituted for amino acids with lower efficiency. Oocytes were refractory to changes in external amino acid concentration unless surface permeability of the cell to amino acids was increased by overexpression of the System L amino acid transporter. Amino acid-induced, rapamycin-sensitive activation of p70^S6K^ was conferred when System L-expressing oocytes were incubated in extracellular amino acids, supporting intracellular localization of the putative amino acid sensor. In contrast to lower eukaryotes such as yeast, which possess an extracellular amino acid sensor, our findings provide the first direct evidence for an intracellular location for the putative amino acid sensor in animal cells that signals increased amino acid availability to TOR/p70^S6K^.

There is growing evidence that nutrients, in particular amino acids (AAs), exert powerful modulatory effects on proteins involved in cell signaling, mRNA translation, and amino acid transport (for review, see Refs. 1–3). Recent studies indicate that signaling pathways responsive to AAs in mammalian cells show a similarity to those that have already been well characterized in lower organisms such as yeast and bacteria (4–6). The serine-threonine protein kinase mammalian target of rapamycin (/mTOR/) is proposed to act as a “nutrient-dependent gatekeeper” (5) for several cell functions. Two key translational regulators, p70 S6 kinase (p70^S6K^) and eukaryotic initiation factor 4E-binding protein (4E-BP1) lie downstream of /mTOR/ in a cell signaling pathway responsive to AA supplementation (7–10). Increased phosphorylation of p70^S6K^ and 4E-BP1 in response to AA supplementation is inhibited by the immunosuppressant rapamycin, a specific inhibitor of /mTOR/ (5, 6). The activation of p70^S6K^ by phosphorylation enables it to phosphorylate its downstream target ribosomal protein S6 and increase translation of so-called 5’-terminal tract of pyrimidines mRNAs, which generally encode ribosomal proteins and elongation factors (2, 11). The activation of p70^S6K^ is thought to lead to up-regulation of ribosome biosynthesis and thus increase the translational capacity of the cell. Phosphorylation of 4E-BP1 increases protein synthesis by releasing eukaryotic initiation factor 4E, which can then form the eukaryotic initiation factor 4F complex as a prerequisite for translation of the capped mRNAs (reviewed in Ref. 2). Although potential upstream roles of phosphoinositide 3-kinase (8, 9, 12), protein kinase B (7, 8, 12–14), and MAPK (8, 12, 14) in the AA-induced stimulatory effects on 4E-BP1 and p70^S6K^ have all been investigated, their relative contributions remain unclear and may vary depending on cell type and experimental conditions used.

Despite recent attention to the processes of nutrient-related signaling in animal cells (1), a major unanswered question is how these nutrient stimuli are “sensed” by cells to initiate signaling. Does sensing of amino acids occur extracellularly, *e.g.* via a membrane bound receptor or amino acid transporter or intracellularly? This question has important implications for the way in which animal cells respond to changes in nutrient availability, because cytosolic and extracellular amino acid pools may be far from equilibrium.

In an attempt to address these key questions, we have investigated AA sensing in the *X. laevis* oocyte. *X. laevis* oocytes are large single cells of up to 1 mm in diameter, which possess active translational and transcriptional machinery, and are suitable for microinjection of substances (*e.g.* of nutrients in solution) and overexpression of proteins (*e.g.* nutrient transporters). In comparison to typical mammalian cell culture systems, which are necessarily grown in nutrient-rich media making it more difficult to study AA-sensing mechanisms, stage VI oocytes can survive in nutrient-deprived media for several days if necessary and are arrested in meiotic prophase. Nevertheless, they receive a maternal nutrient supply *in vivo* and may be predicted to respond to nutrients including AAs in a manner.

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‡ The abbreviations used are: AA(s), amino acid(s); AOAA, aminooxyacetic acid; BCH, 2-amino-bicyclo[2,2,1]hepta-2-carboxylic acid; MMB, modified Barth’s medium; (m)TOR, (mammalian) target of rapamycin; p70^S6K^, p70 S6 kinase; MAPK, mitogen activated protein kinase; PMA, phorbol 12-myristate 13-acetate; 4E-BP1, eukaryotic initiation factor 4E-binding protein; mRNA, messenger RNA; cRNA, complementary RNA; KIC, α-ketoisocaproic acid; ERK, extracellular signal-regulated kinase.
similar to mammalian cells. Therefore, *X. laevis* oocytes represent a useful experimental system in which we could accurately manipulate intracellular as well as extracellular AA concentrations within physiologically relevant limits to study AA sensing mechanisms using p70<sub>S6K</sub> activity as a downstream reporter. The high sequence identity between *X. laevis* p70<sub>S6K</sub> and mammalian (rat) p70<sub>S6K</sub> (93%) plus conservation of all the phosphorylation sites and regulatory motifs present in the mammalian enzyme (15) supported the use of antibodies developed for phosphorylation sites and regulatory motifs present in the mammalian enzyme.

**Table I**

| AA    | Oocyte [AA]<sup>a</sup> | 1× AA<sup>b</sup> | 5× AA<sup>c</sup> | 20× AA<sup>d</sup> | Increase in [AA], after injection of 20× AA mix |
|-------|-------------------------|------------------|------------------|--------------------|-----------------------------------------------|
|       | mm                      | mm               | mm               | mm                 | %                                            |
| Ser   | 0.64                    | 0.24             | 1.21             | 4.86               | 27.7                                         |
| Gln   | 0.21                    | 0.67             | 3.33             | 13.34              | 228.4                                        |
| Glu   | 4.80                    | 0.075            | 0.38             | 1.52               | 1.2                                          |
| Gly   | 0.55                    | 0.41             | 2.04             | 8.16               | 54.5                                         |
| Ala   | 0.85                    | 0.47             | 2.35             | 9.42               | 40.3                                         |
| Leu   | 0.25                    | 0.16             | 0.80             | 3.22               | 46.4                                         |
| Phe   | 0.17                    | 0.055            | 0.27             | 1.08               | 62.3                                         |
| His   | 0.27                    | 0.07             | 0.34             | 1.36               | 20.3                                         |
| Arg   | 0.47                    | 0.13             | 0.66             | 2.64               | 20.7                                         |

<sup>a</sup> Calculated from data (17) using an estimated oocyte volume of 0.75 μl. Mean increase in individual intracellular AA concentration following microinjection of 27.4 nl of 20× AA/oocyte = 48.5 ± 14.0% (calculated for the 15 AAs where intracellular concentration of AA is known (17)). Complete composition of 1× AA mix is given in text, i.e., intracellular.

<sup>b</sup> Comparable to *X. laevis* plasma AA concentrations (17).

**EXPERIMENTAL PROCEDURES**

**Chemicals**—Unless otherwise specified, chemicals were obtained from Sigma. d-[3H]sucrose, [3H]tryptophan, [3H]glutamine, and [3H]phenylalanine were all purchased from PerkinElmer Life Sciences. *Xenopus laevis Oocyte Isolation*—Oocytes were isolated by collagenase treatment of ovarian tissue obtained from mature female *X. laevis* toads (South African Xenopus facility) using methods described previously (16). Defolliculated, stages V–VI (prophase-arrested) oocytes were selected and maintained at 18°C for 30 min unless stated otherwise prior to lysis in extraction buffer (10 mM HEPES, pH 7.4 containing (in mM) 88 NaCl, 1 KCl, 2.4 NaHCO<sub>3</sub>, 0.82 MgSO<sub>4</sub>, 0.75 CaCl<sub>2</sub>, 0.66 NaNO<sub>3</sub>, 0.5 Hepes, pH 7.6 (adjusted with Tris base), and 10 mg/ml gentamycin sulfate.

**Oocyte Microinjection and Incubations**—Solutions of specified composition (see Figs. 1–7) were microinjected into the cytoplasm of individual oocytes (10–20 oocytes/condition). Composition of the AA mix used is described below (for review see Ref. 17 and Table I). In parallel, separate oocytes were injected with an identical volume of d-[3H]sucrose solution, and the retained injectate volume was calculated from measurement of oocyte radioactivity using methods described previously (16). This volume was 27.4 nl/oocyte. Individual oocytes were co-injected with 4F2hc and IU12 cRNA (50 ng of each cRNA in 50 nl of injected cRNA/oocyte). Oocytes were incubated in MBM for 2–4 days to allow expression of injected cRNA before experimentation.

**Measurement of AA Uptake**—AA transport in oocytes at 2–4 days post-injection was measured at 22°C as influx of radiolebelled AA tracer. Experiments were carried out in MBM buffer, and uptake of 3H-labelled Phe, Gin, or Trp was measured using a method described previously (19).

**Preparation of Cleared Lysates**—Total oocyte lysates were centrifuged at 14,000 × g for 2 min, and the clear supernatant was retained (cleared lysate). The protein concentrations of cleared lysates were determined using the BCA method (Pierce).

**Western Blot Analysis**—Proteins from cleared lysates (40 μg of protein/lane) were resolved by SDS-PAGE (20) and transferred electrophoretically onto nitrocellulose membranes (Amersham Biosciences, Inc.) as described previously (21). Membranes were probed using antibodies specific for p70<sub>S6K</sub> (Santa Cruz Biotechnology, Inc.), phosphorylated p70<sub>S6K</sub> (Thr-389, from Cell Signaling Technologies), phosphorylated p44/42 MAPK (Thr-202/Tyr-204, from Cell Signaling Technologies), and phosphorylated S6 ribosomal protein (Ser-235, kindly supplied by the Division of Signal Transduction Therapy, School of Life Sciences, University of Dundee) and were immunodetected by ECL (Amersham Biosciences, Inc.) using appropriate horseradish peroxidase-conjugated secondary antibodies (Diagnósticos Scotland, Carlake, Lanarkshire, Scotland). Blots were quantified using a Bio-Rad GS-670 imaging densitometer.

**RESULTS**

**Increased Intracellular but Not Extracellular AAs Result in a Rapid TOR-dependent Phosphorylation of p70<sub>S6K</sub> and S6 in Oocytes**—Microinjection of oocytes with 20× total AAs that raises intracellular AA concentrations by small but physiologically relevant amounts (see Table I), produced a mobility shift in p70<sub>S6K</sub> as detected by immunoblot analysis. This well characterized feature reflects phosphorylation of the p70<sub>S6K</sub> protein (Fig. 1A). This increase in p70<sub>S6K</sub> phosphorylation was not specific to phospho- or osmotic effects of the injectate, because it was not observed in oocytes injected with either water or solutions of L-glucose at concentrations of up to 100 mM in the injectate (Fig. 1A and Fig. 3). The intracellular AA-induced increase in p70<sub>S6K</sub> phosphorylation was rapid, being detected within 20
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Fig. 1. Increased intracellular AA concentration in *X. laevis* oocytes results in increased phosphorylation of p70S6K, whereas extracellular AAs have no effect. A, effect of microinjection of AAs on p70S6K. *X. laevis* oocytes (uninjected, U) were microinjected with 20× AA mix or water (W). Following injection, cells were incubated in MBM for either 30 min or 3 h prior to lysis. Cell lysates were subjected to immunoblot analysis using a p70S6K-specific antibody. Retardation of mobility during SDS-PAGE (upward bandshift shown by arrowheads) is a well recognized feature of phosphorylated forms of p70S6K (p70S6K(PP)). B, time course of p70S6K phosphorylation following AA microinjection. Quantification using image densitometry of the increased phosphorylation of p70S6K (appearance of retarded protein band on immunoblot) following microinjection of AAs (20×) or water lysed at various time points post-injection. C, a lack of effect of extracellular AAs on p70S6K. *X. laevis* oocytes were incubated in MBM ± 5x AA mix ± rapamycin (R) for either 1 or 24 h. Cell lysates were prepared and subjected to immunoblot analysis using a p70S6K-specific antibody.

min after injection (Fig. 1B), and was sustained up to 3 h post-injection (Figs. 1A and 1B). In contrast, the incubation of oocytes with excess extracellular total AAs (5× plasma concentration) for either 1 or 24 h resulted in no significant increase in phosphorylation of p70S6K, visualized by the lack of any significant mobility shift of p70S6K following immunoblot analysis (Fig. 1C). Phosphorylation of p70S6K following microinjection of AAs was confirmed using a phospho-specific p70S6K antibody (Fig. 2), and as an index of intracellular p70S6K activity, we studied the phosphorylation of its substrate S6 using an appropriate phospho-specific (Ser-235) antibody (Fig. 2). The phospho-specific p70S6K antibody we have used detects the phosphorylation of Thr-389, which has previously been demonstrated to be a rapamycin-sensitive/mtTOR-dependent phosphorylation site (22, 23). The incubation of oocytes with rapamycin prior to and following microinjection with AAAs prevented the AA-induced mobility shift of the p70S6K protein. In line with this finding, rapamycin also prevented the AA-induced phosphorylation of p70S6K, detected using the phospho-specific antibody, and substantially reduced the concomitant phosphorylation of S6 (Fig. 2). Therefore, it appears that a direct increase in intracellular AA concentrations to ~48% above that normally present within the oocyte (see Table U) results in a TOR-dependent increase in phosphorylation of p70S6K and that of its downstream target ribosomal protein S6, whereas exposure to relatively high concentrations of extracellular AAs (5× plasma levels) had no such effect on this pathway. The observation that the *X. laevis* oocyte is acutely sensitive to increases in internal but not external AA concentration suggests that this cell type possesses an intracellular AA sensor.

Microinjection of L-Leu (20× plasma concentration) alone was sufficient to induce p70S6K phosphorylation and pathway activation over the same timescale (Fig. 2). L-Leucinamide was able to produce a similar effect to l-Leu, but d-Leu was ineffective (Fig. 3A). Certain l-AAAs were able to significantly increase the phosphorylation of p70S6K when microinjected into *X. laevis* oocytes alone at 20× plasma concentration such as Trp (see below), Phe, Arg, Lys, and Gly but not Gln, Glu, Pro, or Ala (for example, see Fig. 3B).

A Relatively Small Increase in Intracellular Leu Concentration Is Sufficient to Result in Increased Phosphorylation of p70S6K—As Leu by itself induced increased phosphorylation of p70S6K, we explored the dose dependence of these effects by microinjecting various concentrations of Leu (Fig. 4). Relatively small increases in intracellular Leu concentration resulted in increased phosphorylation of p70S6K (e.g. injecting 1 mM Leu results in an ~7% increase in intracellular Leu concentration and appears to induce nearly 50% maximal increase in p70S6K phosphorylation, see Fig. 4 and Table I).

Overexpression of a System L AA Transporter Enhances Sensitivity of Oocytes to External AAs—The large size and low surface area/volume ratio of the *X. laevis* oocyte compared with mammalian cells in addition to a relatively low surface permeability to AAs means that intracellular AA concentrations rise relatively slowly as a result of any increase in extracellular AA concentrations (17). Overexpression of the System L AA transport system (24) in the oocyte plasma membrane (25) markedly increases surface permeability to many neutral AAs including Leu, Trp, and Phe, causing for example an 11× increase in [3H]Trp uptake (Fig. 5C) and a 16× increase in [3H]Phe uptake (results not shown) at 1× AA mix concentrations compared with control oocytes. For System L-overexpressing oocytes incubated in 1× AA mix, we estimate that this represents a potential to increase intraoocyte concentrations of AAs such as Phe by up to 75% after 30 min (values approximating those estimated for typical mammalian cells, for review see Ref. 26). Thus, this possibility offers an opportunity to test further the idea that AA sensing is intracellular. When cells overexpressing System L were incubated for 30 min in either plasma AA mix (1 or 20×, Fig. 5, A and B) or 1× Leu (Fig. 5D), there was now an increase in p70S6K phosphorylation that was not seen in similarly treated control cells or in System L-expressing cells incubated in MBM alone (Fig. 5, A and B). As observed for cells microinjected directly with AAs, this increase in p70S6K phos-
phorylation was also inhibited by rapamycin, again implicating TOR-dependent signaling in the AA-induced response (Fig. 5, A and D). Rapamycin (Fig. 5C) or Me2SO (vehicle for rapamycin stock, results not shown) had no significant effect upon Trp uptake by oocytes overexpressing System L.

To confirm that the effects of overexpressed System L AA-transporter were a response to cellular AA entry, oocytes were incubated with 1/1000 plasma AAs plus 5 mM 2-amino-bicyclo[2,2,1]hepta-2-carboxylic acid (BCH), a specific inhibitor of System L-dependent transport (24). 5 mM BCH almost completely inhibited the activity of overexpressed System L transport measured as uptake of [3H]Trp at physiological concentration (Fig. 5C). BCH abolished the ability of external AA to increase p70S6K phosphorylation (Fig. 5B), presumably because it is blocking AA entry into the cells through System L (Fig. 5C) and preventing any subsequent sensing and signaling to TOR/p70S6K (Fig. 5B). BCH microinjected into oocytes had no effect itself upon the phosphorylation of p70S6K (Fig. 6A).

Effects of AA Alcohols, α-Ketosioappric Acid (KIC), and Aminoxyacetic Acid (AOAA) on p70S6K Phosphorylation in X. laevis Oocytes

Yeast cells are believed to sense a decrease in intracellular AA concentration as a consequence of a decrease in tRNA charging (27–29). In this study, the injection of excess

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had no effect upon p70 S6K phosphorylation (data not shown).

As leucinamide proved an effective substitute for Leu as an activator of the p70 S6K pathway in X. laevis oocytes (Fig. 3A), we also examined the effect of KIC, the natural aminotransferase product of Leu, resulting in increased phosphorylation of p70 S6K, although their effects were less potent than those of the respective AAs (Fig. 6B). In contrast, alaninol like Ala itself was without effect (data not shown).

FIG. 6. Effects of BCH, AA alcohols, KIC, and AOAA on p70S6K phosphorylation in X. laevis oocytes. Oocytes were injected with water (W) or solutions specified below. Cell lysates were prepared 30 min post-injection and subjected to immunoblot analysis using a phospho-specific p70 S6K (Thr-389) antibody. A, X. laevis oocytes were microinjected with 5 or 100 mM BCH (B) or 5 or 50 mM Leu (L). B, X. laevis oocytes were microinjected with 3.2 mM Leu (L), 3.2 and 10 mM leucinol (LOL), 1.4 mM Trp (T), and 1.4 and 10 mM tryptophanol (TOL). C, X. laevis oocytes were microinjected with 20× AAs, α-ketoisocaproic acid (K, 100 mM) or ammonium acetate (AO, 100 mM). D, X. laevis oocytes were microinjected with 10 mM LOL + AOAA (100 mM), 10 mM TOL + AOAA (100 mM), or AOAA (100 mM) alone.

In an attempt to isolate any contribution of intracellular KIC-Leu interconversions to the observed response, the effects of microinjecting AOAA, an inhibitor of aminotransferases, were also investigated. Microinjection of AOAA alone (100 mM) in injectate producing an estimated 3.6 mM final concentration in cytosol) had no effect on basal p70 S6K phosphorylation, but it inhibited the stimulatory effects of co-injected KIC, Leu, or AA mix on p70 S6K phosphorylation (Fig. 6C). AOAA also unexpectedly blocked the stimulatory effects of injected leucinol and tryptophanol on p70 S6K phosphorylation (Fig. 6, C and D). To exclude the possibility that AOAA was exerting a general toxic effect on oocytes, we demonstrated that the presence of AOAA had no effect on α-amanitin, indicating an important role for the TOR pathway in this response to AA as also reported for mammalian cells. In a number of mammalian cell types, (m)TOR has been demonstrated to play a pivotal role in AA-sensitive signaling responses, acting as a checkpoint for integration of detected changes in AA availability and signaling to proteins involved in regulation of mRNA translation such as 4E-BP1 and p70 S6K.

In X. laevis oocytes, it appears that intracellular rather than extracellular AA levels must increase to stimulate phosphorylation of p70 S6K, implying the existence of an intracellular mechanism that renders the cell more sensitive to extracellular AA concentrations only when their surface permeability to AAs such as Leu was greatly increased by the overexpression of a System L transporter. It is the increased capacity to transport AAs into the cell via System L rather than the presence of the transporter protein itself that renders the cell more sensitive to extracellular AAs, because cells overexpressing the System L transport system showed no detectable changes in p70 S6K phosphorylation. Because of the low AA transport capacity in native X. laevis oocytes, extracellular AAs will enter the cell at a much slower rate than in cells overexpressing an AA transport system, or indeed cells in which AAs are microinjected directly into the cytoplasm.

The increase in intracellular AA or Leu levels necessary to increase p70 S6K phosphorylation in oocytes is relatively small, indicating that the sensing mechanism can detect small changes above basal values. Indeed, increasing intracellular...

**FIG. 7. Effects of AOAA on activation (phosphorylation) of Erk (p44–42MAPK) and p70 S6K pathways in X. laevis oocytes by leucine and phorbol ester.** Oocytes were injected with L-leucine (3.2 mM) and aminooxyacetate (100 mM) as indicated and then incubated in MBM ± PMA (1 μM) for 30 min prior to lysis. Cell lysates were subjected to immunoblot analysis using phospho-specific antibodies to either p70 S6K (Thr-389) or p44/42 MAPK (Thr-202/Tyr-204).

**DISCUSSION**

The data presented here show that the X. laevis oocyte is able to sense relatively small increases in intracellular AAs such as Leu, resulting in increased phosphorylation of both p70 S6K and its physiological downstream target ribosomal protein S6. The activation of p70 S6K is thought to up-regulate rapidly the translation of 5′-terminal tract of pyrimidine mRNAs encoding proteins involved in mRNA translation, thus increasing the overall capacity of the cell for protein synthesis in response to an increase in AA (substrate) availability. The ability of intracellular AA to activate p70 S6K in oocytes is inhibited by rapamycin, indicating an important role for the TOR pathway in this response to AA as also reported for mammalian cells. The activation of p70 S6K by another stimulus (PMA, see Fig. 7) or (b) activation of the p44/42 MAPK (Erk) pathway (Fig. 7). These results argue for a relatively specific effect of AOAA in our experiments and also show that Leu activation of p70 S6K does not involve activation of the ERK pathway. Therefore, it is possible that the observed effects of AOAA result from it inhibiting the putative intracellular AA sensor rather than through inhibition of aminotransferases of which AA alcohols are not substrates to our knowledge.

To investigate further the possible involvement of tRNA aminoacylation in regulation of p70 S6K, we microinjected three competitive inhibitors of aminoacyl-tRNA charging by tRNA synthetases (30). Surprisingly, increased intracellular concentrations of leucinol and tryptophanol resulted in increased phosphorylation of p70 S6K, although their effects were less potent than those of the respective AAs (Fig. 6B). In contrast, alaninol like Ala itself was without effect (data not shown).
Leu concentration by only ~7% (Fig. 4) produces a detectable increase in the phosphorylation of p70S6K compared with uninjected cells or cells injected with water. Such increases in intracellular AA concentration are well within physiological limits. For example, in rats that have been fasted for 60 h, re-feeding with protein results in increases in muscle and liver Leu concentrations of 81 and 85%, respectively, within 1 h of the meal (26).

Our findings raise the question of the nature of the putative AA intracellular sensor(s). Are AAs detected directly by an AA receptor, or are indirect indices of AA concentration (e.g. transaminase activity, tRNA charging, see below) monitored by the systems triggering nutrient-sensitive signaling pathways? A transmembrane AA receptor (Slay1p) has been characterized in yeast (31), but no structural homologue has been identified to date in higher organisms. In rat hepatocytes, however, Miotto et al. (32) have shown that the antigen peptide derivative Leu-8-multiple antigen peptide, which is not transported into hepatocytes, was as effective as Leu at suppressing macroautophagy and proteolysis. On this basis, they proposed site(s) on the plasma membrane that can generate intracellular signals in response to stimulation by extracellular Leu or Leu analogues (32). More recently, Conigrave et al. (33) have suggested that the functional properties of a Ca2+-receptor reveal a potential role as a sensor for AAs. However, the effects of Ca2+-receptor activation by AAs on downstream signaling targets (e.g. TOR, 4E-BP1, or p70S6K) have not been investigated (33). Ca2+-receptor was found to be more sensitive to aromatic AAs than branched chain AAs. Leu, for example, was found to be ineffective in activating this sensor. This observation contrasts with our results and those of others (7, 8) showing that certain AA alcohols and leucinamide as well as AAs stimulated p70S6K in Jurkat cells. One possible explanation for this difference with our results and those of others (7, 8) showing that...