GLUT1 enhances mTOR activity independently of TSC2 and AMPK

Carolyn L. Buller,² Charles W. Heilig,³ and Frank C. Brosius, 3rd¹²

Departments of ¹Internal Medicine and ²Molecular and Integrative Physiology, University of Michigan Medical School, Ann Arbor, Michigan; and ³Department of Medicine, University of Florida, Jacksonville, Florida

Submitted 13 August 2010; accepted in final form 24 May 2011

Buller CL, Heilig CW, Brosius FC 3rd. GLUT1 enhances mTOR activity independently of TSC2 and AMPK. Am J Physiol Renal Physiol 301: F588–F596, 2011. First published May 25, 2011; doi:10.1152/ajprenal.00472.2010.—Enhanced GLUT1 expression in mesangial cells plays an important role in the development of diabetic nephropathy by stimulating signaling through several pathways resulting in increased glomerular matrix accumulation. Similarly, enhanced mammalian target of rapamycin (mTOR) activation has been implicated in mesangial matrix expansion and glomerular hypertrophy in diabetes. We sought to examine whether enhanced GLUT1 expression increased mTOR activity and, if so, to identify the mechanism. We found that levels of GLUT1 expression and mTOR activation, as evidenced by S6 kinase (S6K) and 4E-BP-1 phosphorylation, changed in tandem in cell lines exposed to elevated levels of extracellular glucose. We then showed that increased GLUT1 expression enhanced S6K phosphorylation by 1.7- to 2.9-fold in cultured mesangial cells and in glomeruli from GLUT1 transgenic mice. Treatment with the mTOR inhibitor, rapamycin, eliminated the GLUT1 effect on S6K phosphorylation. In cells lacking functional tuberous sclerosis complex (TSC) 2, GLUT1 effects on mTOR activity persisted, indicating that GLUT1 effects were not mediated by TSC. Similarly, AMP kinase activity was not altered by enhanced GLUT1 expression. Conversely, enhanced GLUT1 expression led to a 2.4-fold increase in binding of mTOR to its activator, Rheb, and a commensurate 2.1-fold decrease in binding of Rheb to gyceraldehyde 5-phosphate dehydrogenase (GAPDH) consistent with mediation of GLUT1 effects by a metabolic effect on GAPDH. Thus, GLUT1 expression appears to augment mesangial cell growth and matrix protein accumulation via effects on glycolysis and decreased GAPDH interaction with Rheb.

DIABETIC NEPHROPATHY is the most common cause of end-stage renal disease in the United States (33). Many factors have been implicated in its pathogenesis; however, the critical mechanisms remain incompletely understood. The earliest manifestations of diabetic nephropathy occur in the kidney glomerulus and, therefore, most attention to mechanisms has focused on altered responses of diabetic glomerular cells, including mesangial cells. Expression of the facilitative glucose transporter, GLUT1, is increased in glomeruli of diabetic rats (35) and mice (5) and in the renal cortex and renal tubule segments of diabetic mice (6, 25).

An increase in GLUT1 expression, either in cultured mesangial cells (11) or kidney glomerular cells in vivo (39), leads to signaling alterations that are found in mesangial cells exposed to high-glucose concentrations or to glomeruli from diabetic animals. These changes include activation of protein kinase C isoforms, transforming growth factor β, NF-κB, and aldose reductase, as well as increases in vascular endothelial growth factor and extracellular matrix production (11, 34, 39, 40). Conversely, reduction of GLUT1 expression in mesangial cells exposed to high glucose (12) and in diabetic mice prevents such alterations (5). Increases in GLUT1 expression result in major enhancement of glucose utilization in mesangial cells (11, 34). Thus, it appears that the number of GLUT1 transporters, rather than extracellular glucose concentrations per se, regulates mesangial cell glucose metabolic flux.

There is increasing evidence that the mammalian target of rapamycin (mTOR) and its downstream effector, p70 S6 kinase 1 (S6K), play significant roles in the pathogenesis of diabetic nephropathy (32). In animal models of diabetes, there is increased glomerular mTOR activation and S6K phosphorylation and activation (32). Moreover, treatment of diabetic animals with rapamycin, a mTOR-specific inhibitor, ameliorates glomerular hypertrophy and mesangial expansion in diabetic animals (26, 32, 36, 42). mTOR functions in two distinct multi-protein complexes, mTORC1 and mTORC2 (30, 37). In mTORC1, mTOR associates with mLST8/GβL, raptor and PRAS40, and is sensitive to inhibition by rapamycin. In the mTORC2 complex, mTOR interacts with mLST8/GβL, rictor and Sin1, and is rapamycin insensitive, at least acutely (16, 37, 41). Rheb, a GTP-binding protein modulated by tuberous sclerosis complex (TSC), regulates mTOR activity (9, 17). When bound to GTP, Rheb activates mTOR activity (9, 17). We previously found that GLUT1 expression is enhanced by mTORC1 activation in several cell types (3).

In the current study, we tested the hypothesis that increased GLUT1 expression enhances activation of mTORC1 in mesangial cells. We found that GLUT1 expression enhances mTORC1 activity but that the activation occurs through an unexpected pathway involving GAPDH interaction with Rheb that is modulated by GLUT1 expression, suggesting that mTOR activation by GLUT1 in mesangial cells is an important feature of progressive diabetic glomerulopathy.

MATERIALS AND METHODS

Cell lines. HEK293 cells were obtained from American Type Culture Collection (Manassas, VA). Rat glomerular mesangial cell (MC) lines were developed by Dr. Charles Heilig (University of Florida) and have been previously characterized by our groups (11, 13, 14). The MC lines were grown in RPMI, 20% NuSerum IV and G418. Rat LEF cell lines were derived from spontaneous renal tubular tumors in Long Evans Eker rats (15). Cells from these rats have an inactivating germline mutation in the TSC2 gene (22, 43). LEF-TSC2 cells are stably transfected to express functional TSC2 and have been reported previously (4). LEF ± TSC2 cells were grown in DMEM/F-12 and 10% fetal calf serum ± G418. Appropriate concentrations of mannitol were used in the media to maintain osmolarity in the control plates compared with those with increased extracellular glucose. Mannitol and 2-deoxy-glucose (2-DOG) were purchased from Sigma (St. Louis, MO).
Infection and siRNA studies. A GLUT1 adenoviral construct (AdGT1) and an empty vector control adenoviral construct (Ad-empty) were utilized as previously reported (38). Cells were cultured in conditioned RPMI media for 24 h at 37°C and a multiplicity of infection of 5 was used to infect the cells as previously reported (38). All adenoviral work was done in accordance with the guidelines of the University of Michigan Institutional Biosafety Committee.

Stealth RNAi siRNA Select set of oligonucleotides for GLUT1 (Slc2a1) was purchased from Invitrogen (Carlsbad, CA). Cellular transfection of siRNA oligonucleotides was accomplished using the Silencer siRNA Transfection II kit (Invitrogen) containing Ambion siPORT Amine transfection reagent and negative control oligonucleotides. Experiments were performed in rat mesangial cells according to the kit instructions.

[^H]2-DOG uptake. Cells were grown to ~90% confluence before treatment and 2-DOG uptake analysis. 2-DOG uptakes were performed as previously described (3). Briefly, plates were washed once with Krebs-Ringer phosphate buffer (KRP; in mmol/l: 128 NaCl, 5.2 KCl, 1.3 CaCl2, 2.6 MgSO4, and 10 Na2HPO4) and then incubated with KRP buffer supplemented with 1% BSA for 10–30 min at 37°C. The KRP buffer was removed and replaced with 0.1 mM unlabeled 2-DOG (Sigma) and 0.5 μCi/ml [3H]-2-DOG (Perkin Elmer, Waltham, MA) in KRP buffer + 1% (wt/vol) BSA + 20 mM cytochalasin B, an irreversible inhibitor of glucose transport, at 37°C for 5 min. Previous studies showed that uptake is linear for at least 10 min in the control MC line (34). The plates were subsequently washed twice for 5 min each time with cold KRP solution containing 200 μM phloretin to quench 2-DOG uptake. The samples were then lysed in buffer (10 mM Tris·HCl, pH 7.0, 150 mM NaCl, 1% Triton X-100, and 0.1% SDS). A portion of each sample was used for determination of protein concentration by a bicinchoninic acid assay (Pierce, Rockford, IL), and the rest was utilized for scintillation counting. 2-DOG uptake (in nmol·mg protein⁻¹·min⁻¹) was calculated after correction for non-specific uptake in the presence of cytochalasin B.

Animals. Animals were handled according to the guidelines set forth by the University Committee for the Use and Care of Animals at the University of Michigan. The protocol for these studies was reviewed and approved by the University Committee on the Use and Care of Animals at the University of Michigan. Transgenic mice that overexpress GLUT1 (GT1S mice) were generated as reported (39). Renal glomeruli were isolated for Western blot analysis as previously described (44). Briefly, anesthetized animals were perfused with ice-cold PBS and kidneys were excised, minced, and pressed through a nylon filter into a beaker while being rinsed with ice-cold PBS. A magnet on the bottom of the beaker was used to collect glomeruli containing iron oxide. The glomeruli were transferred to a microcentrifuge tube and resuspended in protein lysis buffer.

Western blotting analysis. Cellular proteins were separated by SDS-PAGE followed by transfer to nitrocellulose membranes. Membranes were blocked with 5% milk in TBS-T for 30 min to an hour, incubated in primary antibody overnight at 4°C and secondary antibody for 1 h. After autoradiography, the films were scanned and quantified using NIH ImageJ. For quantitation, phosphorylated proteins were normalized to the levels of the total (phosphorylated + nonphosphorylated) protein. Quantitation of GLUT1 was accomplished by normalizing to β-tubulin.

Primary antibodies for mTOR, GAPDH, S6K, phosphoS6K (Thr389), 4E-binding protein 1 (4E-BP-1), phospho4E-BP-1 (Thr37/46), AMP kinase (AMPK), phosphoAMPK (Thr172), acetyl CoA carboxylase (ACC), phosphoACC (Ser79), and β-tubulin were ob-

---

Fig. 1. Elevated extracellular glucose induced S6 kinase (S6K) phosphorylation and GLUT1 expression in mesangial cells but reduced them in HEK293 cells. In rat mesangial cells, exposure to high glucose for 24 h led to increased S6K(Thr389) phosphorylation (A; n = 5, **P < 0.01) and GLUT1 protein expression (B; n = 5, *P < 0.05) compared with control. In contrast, in HEK293 cells, incubation in high glucose for 24 h decreased S6K(Thr389) phosphorylation (C; n = 5, *P < 0.05) and GLUT1 expression (D; n = 5, *P < 0.05).
tained from Cell Signaling Technology (Beverly, MA). Rheb (C-19) and secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The GLUT1 antibody was from Dr. Christin Carter-Su (University of Michigan) and has been reported in many of our previous studies (3, 27–29, 38).

**Immunoprecipitation experiments.** These experiments were performed using protein A/G agarose beads from Santa Cruz Biotechnology per the manufacturer’s recommended protocol. In brief, 200 to 2,000 μg of protein were incubated with antibody for 1 h on ice and then incubated with protein A/G beads overnight at 4°C. The beads were washed, and the pellet was resuspended in sample buffer. The samples were then separated by SDSPAGE and transferred to a nitrocellulose membrane. After blocking, the membrane was probed with primary antibodies and then with horseradish peroxidase-conjugated secondary antibodies. The bands were visualized using an enhanced chemiluminescence system.

---

**Fig. 2. GLUT1 levels regulated mammalian target of rapamycin (mTOR) activity.** Adenoviral infection of GLUT1 (AdGT1) in mesangial cells (MClacZ) resulted in increased GLUT1 expression (A; n = 6, *P < 0.05) and increased uptake of the glucose analog 2-deoxyglucose (2-DOG; B; n = 6, **P < 0.01). GLUT1 also led to increased phosphorylation of S6K(Thr389) (C; n = 6, **P < 0.01) and 4E-BP-1 (Thr37/46) (D; n = 6, *P < 0.05) after 24 h compared with control (MClacZ Ad-empty). siRNA knockdown of GLUT1 reduced GLUT1 levels (E; n = 5, *P < 0.05, **P < 0.05) and eliminated the high glucose-induced increase in S6K phosphorylation (F; n = 5, *P < 0.05, **P < 0.05).
were then washed and resuspended in loading buffer before use for Western blot analysis.

Statistical analysis. Prism 4 (Graphpad Software, La Jolla, CA) was used for all statistical analysis. Data were expressed as means ± SE and were analyzed by Student’s t-test or for multiple groups by a one-way ANOVA followed by Tukey post hoc analysis. Differences were considered significant at $P < 0.05$.

RESULTS

Increased extracellular glucose increases S6K phosphorylation in rat mesangial cells. We observed a significant increase in S6K (Thr389) phosphorylation in rat glomerular mesangial cells after incubation in high-glucose media (25 mM) for 24 h, suggesting a significant activation of mTOR (Fig. 1A). A concurrent increase in GLUT1 protein expression was also detected (Fig. 1B), as previously reported (13). In contrast, incubation of HEK293 cells in 25 mM glucose resulted in a significant decrease in both GLUT1 expression and S6K phosphorylation compared with levels in cells incubated in 8 mM glucose (Fig. 1, C and D). Since S6K phosphorylation correlated with GLUT1 and not extracellular glucose levels, we hypothesized that GLUT1 expression, by its effects on glucose utilization, was responsible for the degree of mTOR activation and S6K phosphorylation.

Enhanced GLUT1 expression and glucose uptake augment mTOR activity. Therefore, we examined the effect of short-term GLUT1 overexpression on mTOR activity. We infected control rat mesangial cells (MClacZ) with an adenoviral vector to acutely overexpress GLUT1. GLUT1 levels were increased by 2.4-fold (Fig. 2A) and glucose uptake, as determined by uptake of the glucose analog 2-DOG, was increased 2.3-fold ($P < 0.05$) 24 h after infection in these cells that were maintained in a constant (8 mM) extracellular glucose concentration (Fig. 2B). S6K phosphorylation was increased 2.9-fold at the same time point (Fig. 2C), suggesting that an increase in GLUT1 expression for 24 h was sufficient to augment S6K phosphorylation. Phosphorylation (Thr37/46) of 4E-BP-1, another downstream target of activated mTORC1, was increased by 2.2-fold (Fig. 2D). To further confirm that the high-glucose effects on mTOR activity were due to enhanced GLUT1 expression, we knocked down GLUT1 with a siRNA approach (Fig. 2E). S6K activity was suppressed by GLUT1 knockdown in the cells cultured in 25 mM glucose to levels below those in cells cultured in 8 mM glucose (Fig. 2F), confirming that GLUT1 levels, independent of extracellular glucose, largely determined mTOR activity.

The GLUT1 effect on mTOR activity and S6K phosphorylation was dependent on glucose uptake and metabolism. Cells were incubated with or without 8 mM glucose or 8 mM mannitol as an osmotic control. Removal of glucose eliminated the increase in S6K phosphorylation in cells that acutely overexpressed GLUT1 (Fig. 3). Rapamycin, a specific acute mTOR inhibitor, abrogated the effect of GLUT1 overexpression on S6K phosphorylation (Fig. 4), confirming that mTOR activity was responsible for S6K phosphorylation.

Chronic augmentation of GLUT1 expression results in persistent mTOR activity in vitro and in vivo. To determine the chronic effects of increased GLUT1 expression, like those seen in diabetic nephropathy, on mesangial cell mTOR activity, we examined a stable GLUT1-overexpressing rat mesangial cell line (MCGT1) that has been systematically characterized previously (11–13). These cells exhibited a 1.8-fold increase in S6K phosphorylation compared with the control-transfected stable cell line (Fig. 5B). We also examined GLUT1 transgenic (GT1S) mice that stably overexpress GLUT1 in mesangial cells in the glomerulus (39). We found a 1.7-fold increase in S6K phosphorylation in renal glomeruli isolated from these animals (Fig. 5D).
GLUT1 effects on mTOR activity are independent of TSC2 and AMPK. Loss of TSC2 function leads to a constitutive increase in mTOR activity (18, 19, 24). To test whether suppression of TSC2 activity mediated the effect of GLUT1 on S6K phosphorylation, we used LEF cells that lack functional TSC2 and exhibit increased S6K phosphorylation when cultured under normal conditions (4), as well as LEF cells that have had TSC function restored by stable transfection with a wild-type TSC2 cDNA. After adenoviral overexpression of GLUT1, we observed a significant increase in S6K phosphorylation (Fig. 6) in both cell lines. Although basal S6K phosphorylation was higher in cells lacking functional TSC2, the augmentation induced by GLUT1 overexpression was still observed in these cells. Thus, suppression of functional TSC2 was not the mechanism by which GLUT1 enhances mTOR activity.

We also determined whether AMPK mediates the effects of GLUT1 overexpression on mTOR activity. Although primary regulation of mTOR by AMPK occurs via TSC2, it has been shown that AMPK can also directly regulate mTORC1 via the mTOR subunit raptor (8). We examined AMPK phosphorylation at Thr172, a marker of activated AMPK, in the rat mesangial cells and detected no significant change in phosphorylation after adenoviral GLUT1 overexpression (Fig. 7A). Additionally, phosphorylation of ACC (Ser79), a substrate of AMPK, was not different between GLUT1-overexpressing and control cells (Fig. 7B). Finally, although the AMPK inhibitor compound C increased S6K phosphorylation in both GLUT1-overexpressing and control cells, the difference between the cell types was not altered by the treatment (Fig. 7C). All three findings indicate that the difference in mTOR activity found in GLUT1-overexpressing cells was not due to altered AMPK activity.
GLUT1 effects on mTOR activity are associated with reduced Rheb and GAPDH interaction. An alternate pathway that was recently shown to mediate the effect of glucose metabolism on mTOR activity involves GAPDH, the mTOR activator, and the small GTPase Rheb. GAPDH has been found to bind Rheb and sequester it, thus preventing its activation of mTOR (23). In rat mesangial cells that over-express GLUT1, we found a 2.1-fold decrease in the amount of GAPDH bound to Rheb (Fig. 8 A). This change coincided with a 2.4-fold increase in the amount of mTOR associated with Rheb (Fig. 8 B).

Although acute exposure to high glucose augments glucose uptake in many cell types and temporarily increases mTOR activity via effects on the GAPDH/Rheb complex (23), the reduction in uptake that occurs within 24 h due to reduced GLUT1 levels should mitigate and even reverse such effects. To demonstrate that this is the case, we exposed HEK293 cells to high extracellular glucose levels for 24 h. While Lee et al. (23) showed that exposure of HEK293 cells to increased extracellular glucose for 30 min led to dissociation of GAPDH from Rheb and increased interaction of Rheb with mTORC1,

![Fig. 7. AMP kinase (AMPK) activity was not altered by GLUT1 overexpression. GLUT1 overexpression (AdGT1) in mesangial cells (MClacZ) did not significantly alter AMPK activity as determined by AMPK(Thr172) and acetyl CoA carboxylase (ACC)(Ser79) phosphorylation (A; n = 3, B; n = 4, *P = not significant). Additionally, the increase in S6K phosphorylation with GLUT1 overexpression was not affected by treatment with an AMPK inhibitor (C; n = 6, *P < 0.05).](image)

![Fig. 8. GLUT1 overexpression (AdGT1) resulted in decreased association of GAPDH with Rheb and increased association of mTOR with Rheb. The amount of GAPDH immunoprecipitated by Rheb in mesangial cells (MClacZ) was reduced (A; n = 9, *P < 0.05) by GLUT1 overexpression. In parallel, there was a significant increase in the amount of mTOR immunoprecipitated by Rheb (B; n = 7, **P < 0.01).](image)
we found that a 24-h exposure of HEK293 cells to the same level of elevated extracellular glucose led to opposite results. Specifically, incubation of HEK293 cells in elevated glucose (25 mM) for 24 h significantly increased Rheb-bound GAPDH (Fig. 9A) and decreased the amount of mTOR pulled down by Rheb (Fig. 9B). Thus, high extracellular glucose levels lead to persistent mTOR activation only in cells that maintain or increase GLUT1 levels, such as mesangial cells.

**DISCUSSION**

In this study, we demonstrate that enhanced GLUT1 expression in mesangial cells results in a glucose-dependent increase in mTORC1 activity as indicated by rapamycin-inhibitable phosphorylation of S6K and 4E-BP-1. This is the first time that a direct relationship has been established between GLUT1 expression, glucose metabolism, and the mTOR pathway. We showed that GLUT1 overexpression, via an increase in glucose uptake and without a change in extracellular glucose, results in stimulation of mTOR activity in mesangial cells that, surprisingly, is both TSC2- and AMPK-independent and appears to occur via decreased GAPDH/Rheb binding and increased Rheb/mTOR binding.

These results are consistent with the recent report by Lee et al. (23). Those investigators conducted a search for binding partners of Rheb in cultured HEK293 cells and discovered a novel interaction between Rheb and GAPDH. The investigators went on to show that the increase in mTOR activation in...
the presence of elevated extracellular glucose was independent of TSC and AMPK. Instead, enhanced glycolytic flux led to a substrate-dependent dissociation of GAPDH and Rheb, which in turn led to increased association of Rheb with mTOR and resultant mTOR activation 30 min after the elevation in extracellular glucose (23). In a subsequent publication, Kim et al. (21) demonstrated that the interaction of GAPDH and Rheb was dependent on the availability of glyceraldehyde 3-phosphate. In a series of atomic force microscopy experiments, the authors found that the glycolytic intermediate and GAPDH substrate glyceraldehyde-3-phosphate was sufficient to prevent binding of RhoB to GAPDH in vitro, thus confirming that enhanced glycolytic substrate production would lead directly to reduced interaction of RhoB and GAPDH.

In our study, we found that enhanced GLUT1 expression, independent of changes in extracellular glucose level, led to a persistent reduction in the association of RhoB with GAPDH, an increase in RhoB association with mTOR, and thereby, persistent activation of mTOR. Interestingly, since GLUT1 levels were reduced after 24 h in HEK293 cells (see Fig. 1), the effect of an increase in extracellular glucose concentration on GAPDH and RhoB-induced mTOR activation was transient in this system. Indeed, as we also showed, mTOR activation was actually suppressed after 24 h of exposure to 25 mM glucose in HEK293 cells and coincided with a significant increase in RhoB-bound GAPDH, as shown in Fig. 9, the same system that was used by Lee et al. for their transient experiments. Such suppression in mTOR activity occurred because reduced GLUT1 levels led to a reduction in glucose utilization, independent of extracellular glucose concentration (11, 34), and thereby enhanced RhoB association with GAPDH, reduced RhoB association with mTOR, and inhibited mTOR activation. This presumably protective response did not occur in mesangial cells that were the focus of most of our experiments, nor does it occur in glomeruli in rodent models of diabetic nephropathy (5). These cells and tissues develop a sustained increase in GLUT1 expression in the presence of high extracellular glucose levels (13) and therefore maintain chronic mTOR activation.

While it is clear that mTORC1 activation in response to altered glucose availability is likely to be regulated by multiple pathways, in cultured mesangial cells the effect of GLUT1 overexpression appears to be mediated solely by the GAPDH/Rheb axis. Although chronic induction of GLUT1 expression in glomeruli in mice leads to increased S6K phosphorylation, it is not known which of the pathways is most critical in vivo. Specific experiments designed to modulate AMPK and/or TSC activity may help to elucidate the priority of these different mTOR regulating pathways in vivo.

Over the past few years, a number of studies showed that activation of the mTOR pathway may be important in the development and progression of diabetic kidney disease (1, 2, 7, 10, 20, 31). Nagai et al. (32) showed that increased phosphorylation of S6K is characteristic of early stages of diabetic nephropathy in animal models. There is a significant increase in mTOR activity shortly after induction of type 1 diabetes by streptozotocin injection in animal models (36), which coincides with significant mesangial and glomerular hypertrophy (32). Treatment of diabetic rats and mice with the mTOR inhibitor rapamycin has been shown to reduce albuminuria, mesangial matrix expansion, and glomerular basement membrane thickening (42). Thus, mTOR activity appears to play an important role in the changes of early diabetic nephropathy. Although GLUT1 has effects on many signaling pathways implicated in diabetic nephropathy (2), it seems likely that GLUT1-induced nephropathy occurs in part via its effects on mTOR activity.

We previously showed that increased mTOR activity leads to increased expression of GLUT1 and glucose flux (3). Here, we demonstrate that increased GLUT1 expression in mesangial cells leads to increased glucose flux and mTOR activity, as measured by S6K phosphorylation (Fig. 10). Together, this suggests a feedforward mechanism leading to persistent GLUT1 overexpression and mTOR activation in diabetic glomeruli. Thus, treatments targeting the potentially pathogenic increase in GLUT1 expression as well as those that inhibit mTOR activation may prove highly effective in reducing the progression of diabetic nephropathy.
GLUT1 ENHANCES mTOR ACTIVITY


